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(54) Title: METHODS AND MATERIALS RELATING TO THE FUNCTIONAL DOMAINS OF DNA BINDING PROTEINS

(57) Abstract

Disclosed are DNA sequences encoding novel DNA binding proteins implicated in regulation of early stages of cell growth. Illustratively provided are human and mouse origin DNA sequences encoding early growth regularoty ("Egr") proteins which include "zinc finger" regions of the type involved in DNA binding. Also disclosed is a detailed analysis of the structure and function of the early growth regulatory protein, Egr-1, delineating independent and modular activation, repression, DNA-binding, and nuclear localization activities. Also disclosed are immunological methods and materials for detection of Egr proteins and hybridization methods and materials for detection and quantification of Egr protein related nucleic acids.

METHODS AND MATERIALS RELATING TO THE FUNCTIONAL DOMAINS OF DNA BINDING PROTEINS

Cross-Reference to Related Application

This is a continuation-in-part application of co-pending U.S. Patent Application Serial No. 07/249,584, filed September 26, 1988, the disclosure of which is incorporated herein by reference. The government owns rights in the present invention pursuant to grant number CA 40046 from The National Cancer Institute (*e.g.,* the National Institutes of Health).

Field of the Invention

The present invention relates generally to DNA binding regulatory proteins and more particularly to polynucleotide sequences encoding early growth regulatory proteins possessing histidine-cysteine "zinc finger" DNA binding domains, to the polypeptide products of recombinant expression of these polynucleotide sequences, and to peptides and polypeptides whose sequences are based on amino acid sequences deduced from these polynucleotide sequences.

Background of the Invention

Among the most significant aspects of mammalian cell physiology yet to be elucidated is the precise manner in which growth factors (e.g., hormones, neurotransmitters and various developmental and differentiation factors) operate to effect the regulation of cell growth. The interaction of certain growth factors with surface receptors of resting cells appears to rapidly induce a cascade of biochemical events thought to result in nuclear activation of specific growth-related genes, followed by ordered expression of other genes. Analysis of sequential activation and expression of genes during the transition from a resting state ("G_0") to the initial growing state ("G_1") has been the subject of substantial research. [Lau *et al.*(1987); Sukhatme *et al.*(1987)]

Much of this research has involved analysis of the expression of known genes encoding suspected regulatory proteins (such as the protooncogenes, c-fos and c-myc) following mitogen stimulation. An alternative approach has involved attempts to identify genes activated by mitogenic stimuli through differential screening of cDNA libraries prepared

from resting cells following exposure to serum and specific growth factors. [Lau *et al.* (1985); Cochran *et al.* (1983).]

Of interest to the background of the invention is the continuously expanding body of knowledge regarding structural components involved in the binding of regulatory proteins to DNA. Illustratively, the so-called receptor proteins are believed to bind to DNA by means of zinc ion stabilized secondary structural fingers premised on the folding of continuous amino acid sequences showing high degrees of conservation of cysteines and histidines and hydrophobic residues. [Gehring (1987).] For example, a "zinc finger" domain or motif, present in Xenopus transcription factor IIIA (TF IIIA), as well as the Drosophila Kruppel gene product and various yeast proteins, involves "repeats" of about 30 amino acid residues wherein pairs of cysteine and histidine residues are coordinated around a central zinc ion and are thought to form finger-like structures which make contact with DNA. The cysteine-histidine (or "CC-HH") zinc finger motif, as opposed to a cysteine-cysteine ("CC-CC") motif of steroid receptors, is reducible to a consensus sequence represented as

Cys Xaa_{2-4} Cys Xaa_3 Phe Xaa_5 Lys Xaa_2 His Xaa_3 His *(SEQ. ID. NO: 67)*

wherein C represents cysteine, H represents histidine, F represents phenylalanine, L represents leucine and X represents any amino acid. [Klug *et al* (1987); Blumberg *et al.* (1987); and Schuh *et al.* (1986).]

Primary response or immediate early genes are those genes induced by mitogenic or other stimuli even in the absence of *de novo* protein synthesis and thus constitute the first step in the biochemical cascade resulting in gene activation or polypeptide expression. One such primary response gene, Egr-1, [Sukhatme, *et al.* 1987; Sukhatme, *et al.* 1988] also known as NGFI-A [Milbrandt 1988], Krox24 [Lemaire, *et al.* 1988]), zif268 [Christy, *et al.* 1988], and TIS8 [Lim, *et al.* 1987], is induced transiently and ubiquitously by mitogenic stimuli and also regulated in response to signals that initiate differentiation.

A transcription factor is a regulatory protein that binds to a specific DNA sequence (e.g., promoters and enhancers) and regulates transcription

of an encoding DNA region. Typically, a transcription factor comprises a binding domain that binds to DNA (a DNA binding domain) and a regulatory domain that controls transcription. Where a regulatory domain activates transcription, that regulatory domain is designated an activation domain. Where that regulatory domain inhibits transcription, that regulatory domain is designated a repression domain.

Egr-1 encodes a nuclear phosphoprotein with three zinc finger motifs of the Cys_2His_2 class, suggesting that Egr-1 may mediate growth response by regulating distal gene expression [Cao, *et al.* 1990]. In this respect Egr-1 is like other immediate early transcription factors of the *fos* [Greenberg, *et al.* 1984; Kruijer, *et al.* 1984] and *jun* [Ryseck, *et al.* 1988] families. The Egr-1 protein is known to be localized to the nucleus [Cao, *et al.* 1990; Day, *et al.*, 1990; Waters, *et al.* 1990], to bind to DNA at a site comprising the polynucleotide sequence CGCCCCCGC [Christy, *et al.* 1989; Cao, *et al.* 1990; Lemaire, *et al.* 1990], and to activate transcription through this specific sequence [Lemaire, *et al.* 1990; Patwardhan, *et al.* 1991]. The evolutionary conservation of this gene [Sukhatme, *et al.* 1988], as well as the broad spectrum of induction -- by TPA and growth factors [Lim, *et al.* 1987; Milbrandt 1988; Lemaire, *et al.* 1988; Christy, *et al.* 1988; and Sukhatme, *et al.* 1988], by neuronal stimuli [Sukhatme, *et al.* 1988; Milbrandt 1988; and Cole, *et al.* 1989], by ischemic injury [Oullette, *et al.* 1990; Gilman, *et al.* 1986], and in some contexts in response to differentiation signals [Sukhatme, *et al.* 1988] -- implicates Egr-1 as an important nuclear intermediary in signal transduction.

DNA binding domains of transcription factors are well known in the art. Exemplary transcription factors known to contain a DNA binding domain are the GAL4, c-*fos*, c-*Jun*, lac1, trpR, CAP, TFIID, CTF, Sp1, HSTF and NF-κB proteins. Preferably, a DNA binding domain is derived from the GAL4 protein.

The GAL4 protein is a transcription factor of yeast comprising 881 amino acid residues. The yeast protein GAL4 activates transcription of genes required for catabolism of galactose and melibiose. GAL4 comprises

numerous discrete domains including a DNA binding domain [Marmorstein *et al.* 1992]. The DNA sequences recognized by GAL4 are 17 base pairs (bp) in length, and each site binds a dimer of the protein. Four such sites, similar but not identical in sequence, are found in the upstream activating sequence (UAS_G) that mediates GAL4 activation of the GAL1 and GAL10 genes, for example [Marmorstein *et al.*, 1992].

Of particular interest to the background of the invention is a recent report [Chowdhury *et al.* 1987] relating to an asserted "family" of genes encoding proteins having histidine/cysteine finger structures. These genes, designated "mkr1" and "mkr2", appear to be the first such isolated from mammalian tissue and are not correlated to any early growth regulatory events.

There continues to exist in the art a need for information concerning the primary structural conformation of early growth regulatory proteins, especially DNA binding proteins, such as might be provided by knowledge of human and other mammalian polynucleotide sequences encoding the same. A body of work suggests the modular nature of transcription factors, in which functional domains are structurally independent and able to confer activity on heterologous proteins [Ptashne 1988]. To date, the domains responsible for these functions have not been identified in Egr-1 and Egr-1 proteins. Activation domains, and more recently repression domains, have been demonstrated to function as independent, modular components of transcription factors. Activation domains are not typified by a single consensus sequence but instead fall into several discrete classes: for example, acidic domains in GAL4 [Ma, *et al.* 1987], GCN4 [Hope, *et al.* 1986], VP16 [Sadowski, *et al.* 1988], and GATA-1 [Martin, *et al.* 1990]; glutamine-rich stretches in Sp1 [Courey, *et al.* 1988] and Oct-2/OTF2 [Müller-Immerglück, *et al.* 1990; Gerster, *et al.* 1990]; proline-rich sequences in CTF/NF-1 [Mermod, *et al.* 1989]; and serine/threonine-rich regions in Pit-1/GHF-1 [Theill, *et al.* 1989] all function to activate transcription. The activation domains of *fos* and *jun* are rich in both acidic and proline residues [Abate, *et al.* 1991; Bohmann, *et al.* 1989]; for other

activators, like the CCAAT/enhancer-binding protein C/EBP [Friedman, *et al.* 1990], no evident sequence motif has emerged.

To date the only well characterized repression domain is the alanine-rich sequence in the *Drosophila* gap protein Krüppel [Licht, *et al.* 1990; Zuo, *et al.* 1991]. Other *Drosophila* proteins such as Even-skipped [Han, *et al.*, 1989; Biggin, *et al.* 1992] and Engrailed (Han, *et al.* 1989; Jaynes, *et al.* 1991], and mammalian DNA-binding proteins such as Tst-1/SCIP [Moniku, *et al.* 1990], WT1 [Madden, *et al.* 1991], and YY1/NF-E1/δ [Shi, *et al.* 1991; Harihan, *et al.* 1991; Park, *et al.* 1991] have been shown to act as repressors. Of these, Krüppel, Engrailed, WT1, and YY1/NF-E1/δ have been shown to confer their repression function on a heterologous DNA-binding domain. However, except in the case of Krüppel, the sequences responsible have not been precisely delineated.

Nuclear localization signals (NLS) are generally short stretches of 8 - 10 amino acids characterized by basic residues as well as proline. NLS sequences are retained in the mature protein, may be found at any position as long as it is exposed on the protein surface, and can be present in multiple copies. Proteins enter the nucleus through nuclear pores by a two-step process: the first step is a rapid, signal-dependent binding to the nuclear pore periphery, while the second step is a slower, ATP-and temperature-dependent translocation across the pore [Garcia-Bustos, *et al.* 1991; Silver 1991].

Precedents for the incorporation of nuclear targeting signals within a DNA-binding domain include *fos* [Tratner, *et al.* 1991]; the progesterone receptor, in which the second finger but not the first functions as an NLS [Guiochon-Mantel, *et al.* 1991]; GAL4 [Silver, *et al.* 1984]; and the homeodomain proteins α2 and Pit-1/GHF-1 [Hall, *et al.* 1990; Theill, *et al.* 1989]. If nuclear localization signals and Cys_2His_2 finger domains-- both typified by basic residues-- have co-evolved, NLS sequences may generally be found adjacent to or integrated within zinc finger domains.

Other bipartite nuclear localization signals have been characterized in the polymerase basic protein 1 of influenza virus (PB1) [Nath, *et al.* 1990];

Xenopus protein N1 [Kleinschmidt, *et al.* 1988]; adenovirus DNA-binding protein (DBP) [Morin, *et al.* 1989]; and the yeast repressor α2 which has two nonhomologous signals, a basic NLS found at the N-terminus, as well as a signal located in the homeodomain [Hall, *et al.* 1984, 1990]. Because each α2 signal gives a different phenotype individually, *Hall et al.* suggest that these nonhomologous signals mediate separate steps in nuclear accumulation.

Availability of polynucleotide sequences associated with specific regulatory functions of proteins, such as those discussed above, would make possible the application of recombinant methods to the large scale production of the proteins in procaryotic and eukaryotic host cells, as well as DNA-DNA and DNA-RNA hybridization procedures for the detection, quantification and/or isolation of nucleic acids associated with these and related proteins. Possession of such DNA-binding proteins, and/or knowledge of the amino acid sequences of the same, would allow, in turn, the development of monoclonal and polyclonal antibodies thereto (including antibodies to protein fragments or synthetic peptides modeled thereon) for use in immunological methods for the detection and quantification of early growth regulatory proteins in fluid and tissue samples as well as for tissue specific delivery of substances such as labels and therapeutic agents to cells expressing the proteins. DNA probes based on the polynucleotide sequences for these mammalian early growth regulatory proteins may be of use in detecting gene markers used for the diagnosis of those clinical disorders which are linked to the marker genes.

Brief Summary of the Invention.

In one aspect, the present invention provides an isolated and purified mammalian early growth regulatory polypeptide comprising one or more functional domains. Preferably, the polypeptide comprises the amino acid residue sequence of ***SEQ ID NO: 1 or SEQ ID NO: 2.*** In another aspect, the present invention contemplates a polypeptide comprising a functional domain that performs a function of activation of transcription, repression of transcription, nuclear localization, or polynucleotide binding.

In an alternative aspect, the polypeptide of the present invention comprises one functional domain. Preferably, the polypeptide of the present invention comprises a functional domain that performs the function of activation of transcription of a polynucleotide. More preferred is the polypeptide of the present invention wherein the functional domain comprises the amino acid residue sequence of ***SEQ ID NO: 3 or SEQ. ID NO: 4.***

In another embodiment, the polypeptide of the present invention comprises a functional domain that performs the function of repression of transcription of a polynucleotide. Preferably, the functional domain of the polypeptide represses transcription of a polynucleotide that encodes a mammalian early growth regulatory polypeptide. More preferably, the functional domain of the polypeptide of the present invention comprises the amino acid residue sequence of ***SEQ ID NO: 5.***

In yet another embodiment, the polypeptide of the present invention comprises a functional domain that performs the function of nuclear localization. Preferably, the functional domain of the polypeptide of the present invention comprises the amino acid residue sequence of ***SEQ ID NO: 6 or SEQ ID NO: 7.***

In still another embodiment, the present invention provides an isolated and purified polypeptide comprising a functional domain that performs the function of binding to a polynucleotide. More preferably, the polypeptide of the present invention binds to a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Even more preferably, the polypeptide comprises the amino acid residue sequence of ***SEQ ID NO: 8.***

In an alternative embodiment, the present invention contemplates a polypeptide product of the *in vitro* or *in vivo* expression of a polypeptide-encoding region of a purified and isolated polynucleotide sequence, wherein said polypeptide product is a fusion polypeptide of a mammalian early growth regulatory polypeptide that comprises one or more functional domains, and part or all of a heterologous protein. More preferably, the polypeptide product comprises a fusion of cro-β-galactosidase and Egr-1 amino acid sequences. Still more preferably, the polypeptide product of the invention comprises a fusion of bovine growth hormone and Egr-1 amino acid sequences.

Still another embodiment of the present invention provides an isolated and purified polynucleotide that encodes a mammalian early growth regulatory polypeptide, other than an intact mammalian early growth regulatory protein, the polypeptide of the invention comprising a functional domain performing the function of activation of transcription, repression of transcription, nuclear localization, or polynucleotide binding.

A further aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide, wherein the polypeptide comprises one functional domain performing the function of activation of transcription, repression of transcription, nuclear localization, or polynucleotide binding. The isolated and purified polynucleotide of the invention is preparable by a process comprising the steps of (a) constructing a library of cDNA clones from a cell that expresses said polypeptide; (b) screening the library with a labelled cDNA probe prepared from RNA that encodes said polypeptide; and (c) selecting a clone that hybridizes to the probe. Preferably, the polynucleotide of the present invention is prepared by the method set forth above, and as further exemplified in Examples 1 and 6.

A further aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide comprising a functional domain that activates transcription of a polynucleotide. More preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that activates transcription of a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Even more preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that has the amino acid residue sequence of ***SEQ ID NO: 3 or SEQ ID NO: 4.*** More preferably still, the polynucleotide of the present invention comprises the nucleotide sequence of ***SEQ ID NO: 9 or SEQ ID NO: 10.***

A further aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide comprising a functional domain that represses transcription on a polynucleotide. More preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that represses transcription of a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Even more preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that has the amino acid residue sequence of ***SEQ ID NO: 5***. More preferably still, the polynucleotide of the present invention comprises the nucleotide sequence of ***SEQ ID NO: 11***.

Another aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide comprising a functional domain that performs the function of nuclear localization. More preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that has the amino acid residue sequence of ***SEQ ID NO: 6 or SEQ ID NO: 7***. More preferably still, the polynucleotide of the present invention comprises the nucleotide sequence of ***SEQ ID NO: 12 or SEQ ID NO: 13***.

Another aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide comprising a functional domain that binds to a polynucleotide. More preferably, the polynucleotide of the present invention encodes a polypeptide that comprises a functional domain that binds to a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Even more preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that has the amino acid residue sequence of ***SEQ ID NO: 8***. More preferably still, the polynucleotide of the present invention comprises the nucleotide sequence of ***SEQ ID NO: 14***.

Yet another aspect of the present invention contemplates an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of ***SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ***

ID NO: 14, wherein the polynucleotide hybridizes to a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Preferably, the isolated and purified polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14. For example, the polynucleotide of the invention can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of the disclosed nucleotide sequences.

In yet another embodiment of the present invention, there is provided an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 9. The polynucleotide of the invention hybridizes to SEQ ID NO: 9, or a complement of SEQ ID NO: 9. Preferably, the isolated and purified polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of SEQ ID NO: 9. For example, the polynucleotide of the invention can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of SEQ ID NO: 9.

Alternatively, the present invention contemplates an isolated and purified polynucleotide that comprises a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 10. The polynucleotide of the invention hybridizes to SEQ ID NO: 10, or a complement of SEQ ID NO: 10 Preferably, the polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of SEQ ID NO: 10. For example, the polynucleotide can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of SEQ ID NO: 10

Alternatively, the present invention contemplates an isolated and purified polynucleotide that comprises a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 11. The polynucleotide of the invention hybridizes to SEQ ID NO: 11, or a complement of SEQ ID NO: 11. Preferably, the polynucleotide comprises a

base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 11***. For example, the polynucleotide can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of ***SEQ ID NO: 11***.

In an alternative embodiment, the present invention provides an isolated and purified polynucleotide that comprises a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of ***SEQ ID NO: 12***. The polynucleotide of the invention hybridizes to ***SEQ ID NO: 12*** or a complement of ***SEQ ID NO: 12***. Preferably, the polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 12***. For example, the polynucleotide can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of ***SEQ ID NO: 12***.

In an alternative embodiment, the present invention provides an isolated and purified polynucleotide that comprises a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of ***SEQ ID NO: 13***. The polynucleotide of the invention hybridizes to ***SEQ ID NO: 13***, or a complement of ***SEQ ID NO: 13***. Preferably, the polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 13***. For example, the polynucleotide can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of ***SEQ ID NO: 13***.

In an alternative embodiment, the present invention contemplates an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of ***SEQ ID NO: 14***. The polynucleotide of the invention hybridizes to ***SEQ ID NO: 14***, or a complement of ***SEQ ID NO: 14***. Preferably, the polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 14***. For example, the polynucleotide can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of ***SEQ ID NO: 14***.

In yet another embodiment, the present invention contemplates an expression vector comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that performs the function of activation of transcription. Preferably, the expression vector of the present invention comprises an enhancer/promoter operatively linked to the polynucleotide. More preferably, the expression vector comprises a cytomegalovirus enhancer/promoter operatively linked to the polynucleotide.

In still another embodiment, the present invention contemplates an expression vector comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that performs the function of repression of transcription. Preferably, the expression vector of the present invention comprises an enhancer/promoter operatively linked to the polynucleotide. More preferably, the expression vector comprises a cytomegalovirus enhancer/promoter operatively linked to the polynucleotide .

In another embodiment, the present invention contemplates an expression vector comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that performs the function of nuclear localization. Preferably, the expression vector of the present invention comprises an enhancer/promoter operatively linked to the polynucleotide. More preferably, the expression vector comprises a cytomegalovirus enhancer/promoter operatively linked to the polynucleotide .

In an alternative embodiment, the present invention contemplates an expression vector comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that performs the function of polynucleotide binding. Preferably, the expression vector of the present invention comprises an enhancer/promoter operatively linked to the polynucleotide. More preferably, the expression vector comprises a cytomegalovirus enhancer/promoter operatively linked to the polynucleotide.

Another embodiment of the present invention provides a cell transfected with a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Preferably, the cell is transfected with the polynucleotide of ***SEQ ID NO: 15***. Alternatively, the present invention

provides a cell transfected with the polynucleotide of ***SEQ ID NO: 9 or SEQ ID NO: 10.*** Yet another embodiment contemplates a cell transfected with the polynucleotide of ***SEQ ID NO: 11.*** An alternative embodiment provides a cell transfected with the polynucleotide of ***SEQ ID NO: 12 or SEQ ID NO: 13.*** Another embodiment of the present invention contemplates a cell transfected with the polynucleotide of 14.

The invention also contemplates a process of preparing a mammalian early growth regulatory polypeptide comprising: (a) transfecting a cell with a polynucleotide that encodes the polypeptide to produce a transformed cell; and (b) maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide. The invention still further contemplates a mammalian early growth regulatory polypeptide made by such a process.

The invention alternatively contemplates a process for preparing a polypeptide that comprises a functional domain that activates transcription comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes the polypeptide to produce a transformed cell; and (2) maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide. The invention still further contemplates a polypeptide comprising a functional domain that activates transcription made by such a process.

An embodiment of the present invention provides a process for preparing a polypeptide that comprises a functional domain that represses transcription comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes the polypeptide to produce a transformed cell; and (2) maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide. The invention still further contemplates a polypeptide comprising a functional domain that represses transcription made by such a process.

The invention also contemplates a process for preparing a polypeptide that comprises a functional domain that performs the function of nuclear localization comprising: (1) transfecting a cell with an expression vector

comprising a polynucleotide that encodes the polypeptide to produce a transformed cell; and (2) maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide. The invention still further contemplates a polypeptide that performs the function of nuclear localization made by such a process.

The invention also contemplates a process for preparing a polypeptide that comprises a functional domain that binds to a polynucleotide comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes the polypeptide to produce a transformed cell; and (2) maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide. The invention still further contemplates a polypeptide that comprises a functional domain that binds to a polynucleotide where the polypeptide is made by such a process.

In an alternate embodiment of the invention, transcription on a polynucleotide is activated by a process comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes a polypeptide comprising a functional domain that activates transcription; and (2) maintaining that cell under physiological conditions sufficient to activate transcription.

In another embodiment of the invention, transcription on a polynucleotide is repressed by a process comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes a polypeptide comprising a functional domain that represses transcription; and (2) maintaining that cell under physiological conditions sufficient to repress transcription.

In yet another embodiment of the present invention, nuclear localization in the expression of polypeptides is achieved by a process comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes a polypeptide comprising a functional domain that acts as a nuclear localization signal; and (2) maintaining that cell under physiological conditions sufficient to activate transcription.

In another embodiment of the invention, binding to a polynucleotide is achieved by a process comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes a polypeptide comprising a polynucleotide-binding functional domain; and (2) maintaining that cell under physiological conditions sufficient to repress transcription.

An alternative embodiment of the present invention contemplates a pharmaceutical composition comprising a mammalian early growth regulatory polypeptide and a physiologically acceptable carrier. Yet another embodiment provides a pharmaceutical composition comprising a polypeptide that comprises a functional domain that activates transcription, and a physiologically acceptable carrier. Still another embodiment of the present invention provides a pharmaceutical composition comprising a polypeptide that comprises a functional domain that represses transcription, and a physiologically acceptable carrier.

An embodiment of the present invention also provides a pharmaceutical composition comprising a polypeptide that comprises a functional domain that functions as a nuclear localization signal, and a physiologically acceptable carrier. Alternatively, the present invention provides a pharmaceutical composition comprising a polypeptide that comprises a polynucleotide-binding functional domain, and a physiologically acceptable carrier.

In another embodiment, the present invention provides a pharmaceutical composition comprising a polynucleotide that encodes a mammalian early growth regulatory polypeptide and a physiologically acceptable carrier. Another embodiment provides a pharmaceutical composition comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that activates transcription, and a physiologically acceptable carrier. Alternatively, the present invention contemplates a pharmaceutical composition comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that represses transcription, and a physiologically acceptable carrier.

In yet another embodiment, the present invention contemplates a pharmaceutical composition comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that acts as a nuclear localization signal, and a physiologically acceptable carrier. In still another embodiment, the present invention provides a pharmaceutical composition comprising a polynucleotide that encodes a polypeptide that comprises a polynucleotide-binding functional domain, and a physiologically acceptable carrier.

The invention also contemplates an antibody specifically immunoreactive with a mammalian early growth regulatory polypeptide. Preferably the antibody is a monoclonal antibody. More preferably, the antibody specifically immunoreacts with the amino acid residue sequence: His Leu Arg Gln Lys Asp Lys Lys Ala Asp Lys Ser Lys (***SEQ ID NO: 38***), or with the amino acid residue sequence: Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His Thr Lys Ile (***SEQ ID NO: 39***).

In another aspect, the present invention contemplates diagnostic assay kits for detecting the presence of mammalian early growth regulatory polypeptides in biological samples, where the kits comprise a first container containing a first antibody capable of immunoreacting with mammalian early growth regulatory polypeptides, with the first antibody present in an amount sufficient to perform at least one assay. Preferably, the assay kits of the invention further comprise a second container containing a second antibody that immunoreacts with the first antibody. More preferably, the antibodies used in the assay kits of the present invention are monoclonal antibodies. Even more preferably, the first antibody is affixed to a solid support. More preferably still, the first and second antibodies comprise an indicator, and, preferably, the indicator is a radioactive label or an enzyme.

In an alternative aspect, the present invention provides diagnostic assay kits for detecting the presence, in biological samples, of polynucleotides that encode mammalian early growth regulatory polypeptides, the kits comprising a first container that contains DNA probe molecules that are complementary to a sequence of from about 15 to about

40 contiguous nucleotide bases of ***SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14***, or of any of their complements, wherein the DNA probe molecules are capable of hybridizing to polynucleotides that encode mammalian early growth regulatory polypeptides.

In another embodiment, the present invention contemplates diagnostic assay kits for detecting the presence, in a biological sample, of antibodies immunoreactive with mammalian early growth regulatory polypeptides, the kits comprising a first container containing mammalian early growth regulatory polypeptides that immunoreact with the antibodies, with the polypeptides present in an amount sufficient to perform at least one assay.

In still another embodiment, the present invention contemplates a process of detecting a mammalian early growth regulatory polypeptide comprising immunoreacting the polypeptide with an antibody that is specifically immunoreactive to form a conjugate, and detecting the conjugate.

The invention also contemplates a process of detecting a messenger RNA transcript that encodes a mammalian early growth regulatory polypeptide, which process comprises hybridizing RNA with a polynucleotide sequence that encodes a mammalian early growth regulatory polypeptide. Preferably, the present invention provides a process of detecting a DNA molecule that encodes for a mammalian early growth regulatory polypeptide, which process comprises hybridizing a sample of DNA with a polynucleotide that encodes a mammalian early growth regulatory polypeptide.

In another embodiment, the present invention provides a method of detecting a disease genetically linked to a mammalian Egr gene comprising the step of quantitating polynucleotide sequences encoding a mammalian early growth regulatory polypeptide.

Brief Description of the Drawings

In the drawings, which form a part of the specification:

Figure 1, presented in eight panels designated 1.1-1.8, provides a 3086 base nucleotide sequence (***SEQ ID NO:15***) for a mouse Egr-1 DNA clone as well as a deduced sequence of 533 amino acid residues (***SEQ ID NO: 1***) for the protein;

Figure 2 provides a partial restriction map of Egr-1 DNA clones together with information concerning the position of the protein coding sequence and the locus of amino acids providing for histidine-cysteine zinc fingers.

Figure 3 provides an amino acid sequence alignment of the DNA binding domain of mouse Egr-1 (***SEQ ID NOS: 17-21***) in comparison with a zinc finger consensus sequence (***SEQ ID NO: 16***), with the Drosophila Kruppel sequence (***SEQ ID NOS: 22-29***) and with the "finger 2" sequence of Xenopus TFIIIA protein (***SEQ ID NO: 30***).

Figure 4, presented in eight panels designated 4.1-4.8, provides a 2820 base nucleotide sequence for a human EGR-2 cDNA clone (***SEQ ID NO: 31***) as well as a deduced sequence of 456 amino acids for the protein (***SEQ ID NO: 2***).

Figure 5, presented in two panels designated 5.1-5.2, provides a 1200 base nucleotide sequence of a mouse Egr-1 genomic clone, specifically illustrating the 5' non-transcribed regulatory region thereof comprising bases -935 through +1.

Figure 6 provides a restriction map and organization of the mouse Egr-1 genomic clone mgEgr-1.1 and a comparison to mouse Egr-1 cDNA.

Figure 7 provides a summary of Egr-1 functional domains, including those for repression of activation, nuclear localization, and activation of transcription (***SEQ ID NOS: 33-35***). The repression domain is boxed with a potential phosphorylation site circled. The 5' basic region involved in nuclear localization is underlined. The three zinc fingers of Egr-1 are aligned for comparison with residues conserved amongst Cys_2His_2 zinc finger proteins enclosed. The basic region of Egr-1 is indicated by + symbols.

Each zinc finger is designated by a black bar. The proline/serine/threonine-rich C-terminal domain is indicated P/S/T.

Figure 8 provides a table of internal deletions of specific amino acid sequences in Egr-1 protein.

Figure 9 provides a pictorial summary of transcriptional activity and expression levels of Egr-1 deletion derivatives. Each construct retains the zinc finger domain (stippled area) and all but the internal deletion Δ284-330 have 20 exogenous amino acids inserted in-frame at the N-terminus.

Figure 10 provides a representation of the pSG424 expression plasmid. GAL4 amino acids 1-147 encoding the DNA-binding domain and nuclear localization signal are expressed from the SV40 early promoter (*SEQ. ID NOS: 36 AND 37*).

Figure 11 illustrates results obtained from titration of repression by GAL4-Egr-1(281-314) and GAL4(1-147). Percent repression is the CAT activity of reporter with the specified amount of effector plasmid relative to the activity of the reporter alone.

Figure 12 illustrates the construction of β-galactosidase fusions.

Figure 13 depicts the localization of Egr-1-β-galactosidase fusions.

Detailed Description of the Invention.

I. The Invention.

Operative association of Egr-encoding polynucleotide sequences provided by the invention with homologous or heterologous species expression control sequences, such as promoters, operators, regulators and the like, allows for *in vivo* and *in vitro* transcription to form messenger RNA which, in turn, is susceptible to translation to provide Egr proteins in large quantities. In one DNA expression system, as illustrated in Example 3, Egr-encoding DNA is operatively associated with a bacteriophage T3 or T7 RNA promoter DNA sequence allowing for *in vitro* transcription and translation in a cell free system. Incorporation of novel DNA sequences of the invention into procaryotic and eucaryotic host cells by standard transformation and transfection processes involving suitable viral and circular DNA plasmid vectors is also within the contemplation of the invention and is

expected to provide useful proteins in quantities heretofore unavailable from natural sources. Fragments of DNA encoding Egr polypeptides of the invention have been incorporated into plasmid vectors according to the procedures illustrated in Example 7, resulting in expression by transformed *E. coli* hosts of fusion proteins sharing immunological characteristics of Egr protein. Use of mammalian host cells is expected to provide for such post-translational modifications (e.g., truncation, glycosylation, and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the invention.

Also provided by the present invention are novel polynucleotide sequences involved in regulation of the transcription of Egr encoding polynucleotides, which sequences are expected to have utility in the efficient recombinant expression of Egr proteins as well as proteins encoded by other structural genes. In addition, the polynucleotide sequences may be used as probes to detect the presence or absence of gene markers used for the diagnosis of clinical disorders linked to those gene markers, as illustrated in Example 8.

Novel polypeptide products of the invention include polypeptides having the primary structural conformation (i.e., amino acid sequence) of Egr proteins or fragments thereof, as well as synthetic peptides, and analogs thereof, assembled to be partially or wholly duplicative of amino acid sequences extant in Egr proteins. Proteins, protein fragments, and synthetic peptides and polypeptides of the invention are expected to have therapeutic, diagnostic, and prognostic uses and also to provide the basis for preparation of monoclonal and polyclonal antibodies specifically immunoreactive with Egr proteins and polynucleotides encoding those proteins, on fragments thereof, as well as to provide the basis for the production of drugs for use as competitive inhibitors or potentiators of Egr-1. Preferred protein fragments and synthetic peptides of the invention include those which share at least one continuous or discontinuous antigenic epitope with naturally occurring Egr proteins.

Antibodies of the Invention, as illustrated in Example 4, preferably bind with high immunospecificity to Egr proteins, fragments, and peptides, preferably recognizing epitopes which are not common to other proteins, especially other DNA binding proteins.

Also provided by the present invention are novel procedures for the detection and/or quantification of Egr proteins and nucleic acids (*e.g.*, DNA and mRNA) specifically associated therewith. Illustratively, antibodies of the invention may be employed in known immunological procedures for quantitative detection of Egr proteins in fluid and tissue samples. Similarly, DNA sequences of the invention (particularly those having limited homology to other DNA's encoding DNA binding proteins) may be suitably labelled and employed for the quantitative detection of mRNA encoding the proteins. Information concerning levels of Egr mRNA may provide valuable insights into growth characteristics of cells.

Among the multiple aspects of the present invention, therefore, is the provision of (a) novel purified and isolated Egr-encoding polynucleotide sequences set out in Figures 1, 4 and 5, as well as (b) specific amino acid sequences corresponding to the independent functional domains of Egr-1 proteins responsible for DNA binding, regulation of activation and nuclear localization, as set out in ***SEQ ID NOS. 3, 5, 6, 7, 8 and 15***. Correspondingly provided are viral or circular plasmid DNA vectors incorporating such DNA sequences and procaryotic and eucaryotic host cells transformed or transfected with such polynucleotide sequences and vectors.

Transcription factors are regulatory proteins that binds to a specific DNA sequence (*e.g.*, promoters and enhancers) and regulate transcription of an encoding DNA region. Typically, a transcription factor comprises a binding domain that binds to DNA (a DNA binding domain) and a regulatory domain that controls transcription. Where a regulatory domain activates transcription, that regulatory domain is designated an activation domain. Where that regulatory domain inhibits transcription, that regulatory domain is designated a repression domain.

Activation domains, and more recently repression domains, have been demonstrated to function as independent, modular components of transcription factors. Activation domains are not typified by a single consensus sequence but instead fall into several discrete classes: for example, acidic domains in GAL4 [Ma, *et al.* 1987], GCN4 [Hope, *et al.*, 1986], VP16 [Sadowski, *et al.* 1988], and GATA-1 [Martin, *et al.* 1990); glutamine-rich stretches in Sp1 [Courey, *et al.* 1988] and Oct-2/OTF2 [Müller-Immerglück, *et al.* 1990; Gerster, *et al.* 1990]; proline-rich sequences in CTF/NF-1 [Mermod, *et al.* 1989]; and serine/threonine-rich regions in Pit-1/GHF-1 [Theill, *et al.* 1989] all function to activate transcription. The activation domains of *fos* and *jun* are rich in both acidic and proline residues [Abate, *et al.* 1991; Bohmann, *et al.* 1989]; for other activators, like the CCAAT/enhancer-binding protein C/EBP [Friedman, *et al.* 1990], no evident sequence motif has emerged.

Polypeptides of the present invention, corresponding to expression products of the Egr-1 gene, are bifunctional polypeptides containing regulatory domains that can both activate and repress transcription, as demonstrated through transient transfection assays. These transcription factors possess a robust serine/threonine-rich N-terminal activation domain and a novel repression domain distinct from the alanine-rich sequence shown to be responsible for repression in the *Drosophila* protein Krüppel [Licht, *et al.* 1990].

The polypeptide of the present invention that represents the sequence incorporating the repression domain of Egr-1 is an extremely compact function of thirty-four amino acids (corresponding to amino acid residues 281-314 (*SEQ ID NO: 5*)) that has been highly evolutionarily conserved through zebrafish, the lowest vertebrate in which a homologue of Egr-1 has been identified. The primary sequence of the Egr-1 repressor is not alanine- or glycine-rich as is the case for Krüppel and suggested to be true for SCIP and YY1, and as such represents a transcriptional motif distinct from those observed previously.

The novel polypeptides of the present invention are one of only a small number of transcription factors that contain modular domains capable of regulating transcription both positively and negatively. Other examples include Krüppel [Zuo, *et al.* 1991], YY1/NF-E1/δ [Hahn 1992], and the immediate early factors *fos* and *jun* [Abate, *et al.* 1991]. The ability to work as either an activator or a repressor may be common to immediate early transcription factors to allow for versatility of effector functions. Post-translational modifications or interactions with cell-type specific factors may enable these complex transcription factors to function as either repressors or activators of transcription. Thus, Egr-1 may function differently in the diverse contexts in which it is expressed--immediately in response to growth stimuli in all cell types and with different kinetics and presumably target specificity in response to differentiation cues in some cell lineages.

II. Early Growth Regulatory Polypeptides with a Plurality of Functional Domains.

Polypeptides of the present invention, corresponding to expression products of the Egr-1 gene, are bifunctional polypeptides containing domains that can both activate and repress transcription. These transcription factors possess a robust serine/threonine-rich N-terminal activation domain and a novel repression domain distinct from the alanine-rich sequence shown to be responsible for repression in the *Drosophila* protein Krüppel [Licht, *et al.* 1990].

A. Peptides and Polypeptides.

In one aspect, the present invention provides an isolated and purified mammalian early growth regulatory polypeptide comprising one or more functional domains. Preferably, the polypeptide comprises the amino acid residue sequence of ***SEQ ID NO: 1 or SEQ ID NO: 2***. In another aspect, the present invention contemplates a polypeptide comprising a functional domain that performs a function of activation of transcription, or repression of transcription, or nuclear localization, or polynucleotide binding.

In yet another aspect, the polypeptide of the present invention comprises three or more functional domains, and the functional domains perform functions comprising activation of transcription, repression of transcription, nuclear localization and polynucleotide binding.

In an alternative aspect, the polypeptide of the present invention comprises one functional domain. Preferably, the polypeptide of the present invention comprises a functional domain that performs the function of activation of transcription on a polynucleotide. More preferably, the polynucleotide comprises the polynucleotide sequence of ***SEQ ID NO: 15.*** Even more preferred is the polypeptide of the present invention wherein the functional domain comprises the amino acid residue sequence of ***SEQ ID NO: 3 or SEQ ID NO: 4.***

In another embodiment, the polypeptide of the present invention comprises a functional domain that performs the function of repression of transcription of a polynucleotide. Preferably, the functional domain of the polypeptide represses transcription of a polynucleotide that encodes a mammalian early growth regulatory polypeptide. More preferably, the functional domain of the polypeptide of the present invention comprises the amino acid residue sequence of ***SEQ ID NO: 5.***

In yet another embodiment, the polypeptide of the present invention comprises a functional domain that performs the function of nuclear localization. Preferably, the functional domain of the polypeptide of the present invention comprises the amino acid residue sequence of ***SEQ ID NO: 6 or SEQ ID NO: 7.***

In still another embodiment, the present invention provides an isolated and purified polypeptide comprising a functional domain that performs the function of binding to a polynucleotide. More preferably, the polypeptide of the present invention binds to a polynucleotide that encodes a mammalian early growth regulatory polypeptide. More preferably, the polypeptide comprises the amino acid residue sequence of ***SEQ ID NO: 8.***

In an alternative embodiment, the present invention contemplates a polypeptide product of the *in vitro* or *in vivo* expression, as illustrated in

Example 3, of a polypeptide-encoding region of a purified and isolated polynucleotide sequence, wherein said polypeptide product is a fusion polypeptide of a mammalian early growth regulatory polypeptide that comprises one or more functional domains, and part or all of a heterologous protein. More preferably, the polypeptide product comprises a fusion of cro-β-galactosidase and Egr-1 amino acid sequences (see Example 7). More preferably, the polypeptide product of the invention comprises a fusion of bovine growth hormone and Egr-1 amino acid sequences.

As used herein, the term "polypeptide" means a polymer of amino acids connected by amide linkages, wherein the number of amino acid residues can range from about 5 to about one million. Preferably, a polypeptide has from about 10 to about 1000 amino acid residues and, even more preferably from about 20 to about 500 amino residues. Thus, as used herein, a polypeptide includes what is often referred to in the art as an oligopeptide (5-10 amino acid residues), a polypeptide (11-100 amino acid residues) and a protein (>100 amino acid residues). A polypeptide encoded by an encoding region can undergo post-translational modification to form conjugates with carbohydrates, lipids, nucleic acids and the like to form glycopolypeptides (e.g., glycoproteins), lipopolypeptides (e.g. lipoproteins) and other like conjugates.

Polypeptides are disclosed herein as amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a single letter or a three letter code as indicated in Table 1 below.

TABLE 1

Amino Acid Residue	3-Letter Code	1-Letter Code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Modifications and changes may be made in the structure of a polypeptide of the present invention and still obtain a molecule having like transcription regulation characteristics. For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of transcription activation activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence (or, of course, its underlying DNA coding sequence) and nevertheless obtain a polypeptide with like properties.

In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art [Kyte & Doolittle, *et al.* 1982]. It is known that certain amino acids can be substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar

biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics.

Those indices are given in Table 2, below.

TABLE 2

Amino acid	Index	Amino acid	Index
isoleucine	(+4.5)	tryptophan	(-0.9)
valine	(+4.2)	tyrosine	(-1.3)
leucine	(+3.8)	proline	(-1.6)
phenylalanine	(+2.8)	histidine	(-3.2)
cysteine	(+2.5)	glutamate	(-3.5)
methionine	(+1.9)	glutamine	(-3.5)
alanine	(+1.8)	aspartate	(-3.5)
glycine	(-0.4)	asparagine	(-3.5)
threonine	(-0.7)	lysine	(-3.9)
serine	(-0.8)	arginine	(-4.5)

It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, for example, enzymes, substrates, receptors, antibodies, antigens, and the like. It is known in the art that an amino acid may be substituted by another amino acid having a similar hydropathic index and still obtain a biologically functionally equivalent polypeptide. In such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biologically functionally equivalent peptide or polypeptide thereby created is intended for use in immunological embodiments. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a polypeptide, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e. with a biological property of the polypeptide.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 ± 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent, polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine. The present invention thus contemplates functional equivalents of a polypeptide that activates transcription on a polynucleotide, as set forth above.

A polypeptide of the present invention is prepared by standard techniques well known to those skilled in the art. Such techniques include, but are not limited to, isolation and purification from tissues known to contain that polypeptide, and expression from cloned DNA that encodes such a polypeptide, using transformed cells (see Examples 3 and 11, *infra*).

B. Preparation of Regulatory Polypeptides.

The invention also contemplates a process of preparing a mammalian early growth regulatory polypeptide comprising: (a) transfecting a cell with a polynucleotide that encodes the polypeptide to produce a transformed cell; and (b) maintaining the transformed cell under biological conditions suffi-

cient for expression of the polypeptide. The invention still further contemplates a mammalian early growth regulatory polypeptide comprising one or more functional domains made by such a process.

The invention alternatively contemplates a process for preparing a polypeptide that comprises a functional domain that activates transcription comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes the polypeptide to produce a transformed cell; and (2) maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide. The invention still further contemplates a polypeptide comprising a functional domain that activates transcription made by such a process.

An embodiment of the present invention provides a process for preparing a polypeptide that comprises a functional domain that represses transcription comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes the polypeptide to produce a transformed cell; and (2) maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide. The invention still further contemplates a polypeptide comprising a functional domain that represses transcription made by such a process.

The invention also contemplates a process for preparing a polypeptide that comprises a functional domain that performs the function of nuclear localization comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes the polypeptide to produce a transformed cell; and (2) maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide. The invention still further contemplates a polypeptide that performs the function of nuclear localization made by such a process.

The invention also contemplates a process for preparing a polypeptide that comprises a functional domain that binds to a polynucleotide comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes the polypeptide to produce a transformed cell; and (2) maintaining the transformed cell under biological conditions

sufficient for expression of the polypeptide. The invention still further contemplates a polypeptide that comprises a functional domain that binds to a polynucleotide where the polypeptide is made by such a process.

Any polypeptide can be encoded by an encoding region of a polynucleotide of the present invention. An encoding region can comprise introns and exons so long as the encoding region comprises at least one open reading frame for transcription, translation and expression of that polypeptide. Thus, an encoding region can comprise a gene, a split gene or a cDNA molecule. In the event that the encoding region comprises a split gene (contains one or more introns), a cell transformed or transfected with a DNA molecule containing that split gene must have means for removing those introns and splicing together the exons in the RNA transcript from that DNA molecule if expression of that gene product is desired.

C. Polynucleotides.

A further aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide, wherein the polypeptide comprises a functional domain performing the function of activation of transcription, repression of transcription, nuclear localization, or polynucleotide binding. The isolated and purified polynucleotide of the invention is preparable by a process comprising the steps of (a) constructing a library of cDNA clones from a cell that expresses said polypeptide; (b) screening the library with a labelled cDNA probe prepared from RNA that encodes said polypeptide; and (c) selecting a clone that hybridizes to the probe. Preferably, the polynucleotide of the present invention is prepared by the method set forth above, and as further exemplified in Examples 1 and 6.

As used herein, the term "polynucleotide" means a sequence of nucleotides connected by phosphodiester linkages. A polynucleotide of the present invention can comprise from about 80 to about several hundred thousand base pairs. Preferably, a polynucleotide comprises from about 80 to about 150,000 base pairs. Preferred lengths of particular polynucleotides are set hereinafter.

A polynucleotide of the present invention can be a deoxyribonucleic acid (DNA) molecule or a ribonucleic acid (RNA) molecule. Where a polynucleotide is a DNA molecule, that molecule can be a gene or a cDNA molecule. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), and uracil (U).

A further aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide comprising a functional domain that activates transcription of a polynucleotide. More preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that activates transcription of a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Even more preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that has the amino acid residue sequence of ***SEQ ID NO: 3 or SEQ ID NO: 4***. More preferably still, the polynucleotide of the present invention comprises the nucleotide sequence of ***SEQ ID NO: 9 or SEQ ID NO: 10***.

A further aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide comprising a functional domain that represses transcription on a polynucleotide. More preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that represses transcription of a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Even more preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that has the amino acid residue sequence of ***SEQ ID NO: 5***. More preferably still, the polynucleotide of the present invention comprises the nucleotide sequence of ***SEQ ID NO: 11***.

Another aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide comprising a functional domain that performs the function of nuclear localization. More preferably, the polynucleotide of the present invention encodes a polypeptide comprising a functional domain that has the amino acid residue sequence of ***SEQ ID NO: 6 or SEQ ID NO: 7***. More preferably still, the polynucleotide of

the present invention comprises the nucleotide sequence of ***SEQ ID NO: 12 or SEQ ID NO: 13***.

Another aspect of the present invention provides an isolated and purified polynucleotide that encodes a polypeptide comprising a functional domain that binds to a polynucleotide. More preferably, the polynucleotide of the present invention encodes a polypeptide that comprises a functional domain that binds to a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Even more preferably, the polynucleotide of the present invention encodes a polypeptide that has the amino acid residue sequence of ***SEQ ID NO: 8***. More preferably still, the polynucleotide of the present invention comprises the nucleotide sequence of ***SEQ ID NO: 14***.

Yet another aspect of the present invention contemplates an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of ***SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14***, wherein the polynucleotide hybridizes to a polynucleotide that encodes a mammalian early growth regulatory polypeptide. Preferably, the isolated and purified polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14***. For example, the polynucleotide of the invention can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of the disclosed nucleotide sequences.

In yet another embodiment of the present invention, there is provided an isolated and purified polynucleotide comprising a base sequence that is identical, or complementary, to a segment of at least 10 contiguous bases of ***SEQ ID NO: 9 or SEQ ID NO: 10***. The polynucleotide of the invention hybridizes to ***SEQ ID NO: 9 or SEQ ID NO: 10***, or a complement of ***SEQ ID NO: 9 or SEQ ID NO: 10***. Preferably, the isolated and purified polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 9***. For exam-

ple, the polynucleotide of the invention can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of ***SEQ ID NO: 9***.

Alternatively, the present invention contemplates an isolated and purified polynucleotide that comprises a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of ***SEQ ID NO: 11***. The polynucleotide of the invention hybridizes to ***SEQ ID NO: 11***, or a complement of ***SEQ ID NO: 11***. Preferably, the polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 11***. For example, the polynucleotide can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of ***SEQ ID NO: 11***.

In an alternative embodiment, the present invention provides an isolated and purified polynucleotide that comprises a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of ***SEQ ID NO: 12 or SEQ ID NO: 13***. The polynucleotide of the invention hybridizes to ***SEQ ID NO: 12 or SEQ ID NO: 13***, or a complement of ***SEQ ID NO: 12 or SEQ ID NO: 13***. Preferably, the polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 12 or SEQ ID NO: 13***. For example, the polynucleotide can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of ***SEQ ID NO: 12 or SEQ ID NO: 13***.

In an alternative embodiment, the present invention contemplates an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of ***SEQ ID NO: 14***. The polynucleotide of the invention hybridizes to ***SEQ ID NO: 14***, or a complement of ***SEQ ID NO: 14***. Preferably, the polynucleotide comprises a base sequence that is identical or complementary to a segment of at least 25 to 70 contiguous bases of ***SEQ ID NO: 14***. For example, the polynucleotide can comprise a segment of bases identical or complementary to 40 or 55 contiguous bases of ***SEQ ID NO: 14***.

D. Expression Vectors.

In yet another embodiment, the present invention contemplates an expression vector comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that performs the function of activation of transcription. Preferably, the expression vector of the present invention comprises an enhancer/promoter operatively linked to the polynucleotide. More preferably, the expression vector comprises a cytomegalovirus enhancer/promoter operatively linked to the polynucleotide. (See Example 9.)

In still another embodiment, the present invention contemplates an expression vector comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that performs the function of repression of transcription. Preferably, the expression vector of the present invention comprises an enhancer/promoter operatively linked to the polynucleotide. More preferably, the expression vector comprises a cytomegalovirus enhancer/promoter operatively linked to the polynucleotide.

In another embodiment, the present invention contemplates an expression vector comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that performs the function of nuclear localization. Preferably, the expression vector of the present invention comprises an enhancer/promoter operatively linked to the polynucleotide. More preferably, the expression vector comprises a cytomegalovirus enhancer/promoter operatively linked to the polynucleotide.

In an alternative embodiment, the present invention contemplates an expression vector comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that performs the function of binding to a polynucleotide. Preferably, the expression vector of the present invention comprises an enhancer/promoter operatively linked to the polynucleotide. More preferably, the expression vector comprises a cytomegalovirus enhancer/promoter operatively linked to the polynucleotide. (See Example 9.)

A promoter is a region of a DNA molecule typically within about 100 nucleotide pairs in front of (upstream of) the point at which transcription begins (i.e., a transcription start site). That region typically contains several types of DNA sequence elements that are located in similar relative positions in different genes. As used herein, the term "promoter" includes what is referred to in the art as an upstream promoter region, a promoter region or a promoter of a generalized eukaryotic RNA Polymerase II transcription unit.

Another type of discrete transcription regulatory sequence element is an enhancer. An enhancer provides specificity of time, location and expression level for a particular encoding region (e.g., gene). A major function of an enhancer is to increase the level of transcription of a coding sequence in a cell that contains one or more transcription factors that bind to that enhancer. Unlike a promoter, an enhancer can function when located at variable distances from transcription start sites so long as a promoter is present.

As used herein, the phrase "enhancer/promoter" means a composite unit that contains both enhancer and promoter elements. An enhancer-promoter is operatively linked to a coding sequence that encodes at least one gene product. As used herein, the phrase "operatively linked" means that an enhancer-promoter is connected to a coding sequence in such a way that the transcription of that coding sequence is controlled and regulated by that enhancer-promoter. Means for operatively linking an enhancer-promoter to a coding sequence are well known in the art. As is also well known in the art, the precise orientation and location relative to a coding sequence whose transcription is controlled, is dependent *inter alia* upon the specific nature of the enhancer-promoter. Thus, a TATA box minimal promoter is typically located from about 25 to about 30 base pairs upstream of a transcription initiation site, and an upstream promoter element is typically located from about 100 to about 200 base pairs upstream of a transcription initiation site. In contrast, an enhancer can be located downstream from the initiation site and can be at a considerable distance from that site.

An enhancer-promoter used in a vector construct of the present invention can be any enhancer-promoter that drives expression in a cell to be transfected. By employing an enhancer-promoter with well-known properties, the level and pattern of gene product expression can be optimized. Exemplary and preferred enhancer-promoters are the cytomegalovirus (CMV) promoter, the Rous sarcoma virus (RSV) RSV-1 LTR promoter, the β-actin promoter, the α-antitrypsin promoter, the apo A1 promoter, and the liver fatty acid binding promoter or the albumin promoter.

A coding sequence of an expression vector is operatively linked to a transcription terminating region. RNA polymerase transcribes an encoding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (RNA). Transcription-terminating regions are well known in the art. A preferred transcription-terminating region used in an adenovirus vector construct of the present invention comprises a polyadenylation signal of SV40 or the protamine gene.

A preferred expression vector is an adenovirus vector construct. The use of adenovirus as a vector for cell transfection is well known in the art. Adenovirus vector-mediated cell transfection has been reported for various cells.

An adenovirus vector of the present invention is replication defective. A virus is rendered replication defective by deletion of the viral early gene region 1 (E1). An adenovirus lacking an E1 region is competent to replicate only in cells, such as human 293 cells, which express adenovirus early gene region 1 genes from their cellular genome. Thus, such an adenovirus can kill cells that do not express that early gene product.

In a preferred embodiment, an adenovirus vector used in the present invention is lacking both the E1 and the E3 early gene regions. Techniques for preparing replication defective adenoviruses are well known in the art.

It is believed that any adenovirus vector can be used in the practice of the present invention. Thus, an adenovirus vector can be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material for production of a replication-defective adenovirus vector.

An adenovirus is engineered to contain a coding DNA sequence for use as a vector. Individual DNA sequences such as cDNAs that encode a gene product are inserted into the adenovirus to create a vector construct. In a preferred embodiment, a coding sequence for a transcription-activating polypeptide is introduced or incorporated into an adenovirus at the position from which the E1 coding sequences have been removed. However, the position of insertion within the adenovirus sequences is not critical to the present invention. A coding sequence can also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described previously. Preferably, the E1 region of adenovirus is replaced by the coding DNA sequence or gene.

The resulting adenovirus vector is co-transfected into 293 cells together with a plasmid carrying a complete adenovirus genome to propagate the adenovirus. An exemplary such plasmid is pJM17. Co-transfection is performed in accordance with standard procedures well known in the art. By way of example, 293 cells are cultured in Dulbecco's modified Eagle's medium containing fetal calf serum. Confluent cultures are split the day before calcium phosphate co-transfection of plasmids. After addition of the DNA to the cells, the cells are shocked (e.g., a 15% glycerol shock) to boost transfection efficiency and the cells are overlaid with agar in DMEM containing fetal calf serum, penicillin, streptomycin sulfate, and other antibiotics or antifungal agents as needed. Monolayers are incubated until viral plaques appear (about 5-15 days).

Those plaques are picked, suspended in medium containing fetal calf serum, and used to infect a new monolayer of 293 cells. When greater than 90% of the cells showed infection, viral lysates are subjected to a freeze/thaw cycle and designated as primary stocks. The presence of

recombinant virus is verified by preparation of viral DNA from infected 293 cells, restriction analysis, and Southern blotting. Secondary stocks are subsequently generated by infecting 293 cells with primary virus stock at a multiplicity of infection of 0.01 and incubation until lysis.

The particular cell line used to propagate the recombinant adeno-viruses of the present invention is not critical to the present invention. Recombinant adenovirus vectors can be propagated on, *e.g.*, human 293 cells, or in other cell lines that are permissive for conditional replication-defective adenovirus infection, *e.g.*, those which express adenovirus E1 gene products "in trans" so as to complement the defect in a conditional replication-defective vector. Further, the cells can be propagated either on plastic dishes or in suspension culture, in order to obtain virus stocks thereof.

Other viruses can also be used as expression vectors. Exemplary such viruses are HSV-2, picornavirus, coronovirus, eunyavirus, togavirus, rahbdovirus, retrovirus, vaccinia virus and parvovirus [(*See, e.g.*, Walsh *et al.*, 1992; Sutter *et al.*, 1992 and Huber *et al.*, 1991)]. As discussed with regard to adenoviruses, those viruses would also be altered in such a way as to render them non-pathogenic.

By way of example, a polynucleotide of the present invention can be incorporated into a parvovirus such as the human parvovirus, the adeno-associated virus. Means for incorporating DNA sequences into such a parvovirus are well known in the art [(Walsh *et al.* 1992].

E. Transformed Cells.

Another embodiment of the present invention provides a cell transfected with the polynucleotide of ***SEQ ID NO: 15.*** Alternatively, the present invention provides a cell transfected with the polynucleotide of ***SEQ ID NO: 9 or SEQ ID NO: 10.*** Yet another embodiment contemplates a cell transfected with the polynucleotide of ***SEQ ID NO: 11.*** An alternative embodiment provides a cell transfected with the polynucleotide of ***SEQ ID NO: 12 or SEQ ID NO: 13.*** Another embodiment of the present invention contemplates a cell transfected with the polynucleotide of ***SEQ ID NO: 14.***

Means for transforming or transfecting cells with exogenous polynucleotides such as DNA molecules are well known in the art and include techniques such as calcium-phosphate or DEAE-dextran-mediated transfection, protoplast fusion, electroporation, liposomes, direct microinjection and adenovirus infection [Sambrook, *et al.* 1989].

Means for transfecting a cell are familiar to those skilled in the relevent art. Preferably, a polynucleotide is contained in an expression vector as set forth in the detailed discussion above. A preferred polynucleotide for use in such a process encodes the amino acid residue sequence of ***SEQ ID NO: 3 or SEQ ID NO: 4***. Biological conditions include temperature, pH, osmolality and the like, as is well known in the art. Temperature is from about 20°C to about 50°C. pH is preferably from about a value of 6.0 to a value of about 8.0. Osmolality is preferably from about 200 milliosmols per liter (mosm/L) to about 400 mosm/L. Other biological conditions needed for transfection and expression of an encoded protein are well known.

The most widely used method is transfection mediated by either calcium phosphate or DEAE-dextran. Although the mechanism remains obscure, it is believed that the transfected DNA enters the cytoplasm of the cell by endocytosis and is transferred to the nucleus. Depending on the cell type, up to 20% of a population of cultured cells can be transfected at any one time. Because of its high efficiency, transfection mediated by calcium phosphate or DEAE-dextran is the method of choice for experiments that require translent expression of the foreign DNA in large numbers of cells. Calcium phosphate-mediated transfection is also used to establish cell lines that carry integrated copies of the foreign DNA, which are usually arranged in head-to-tail tandem arrays.

In the protoplast fusion method, protoplasts derived from bacteria carrying high numbers of copies of a plasmid of interest are mixed directly with cultured mammalian cells. After fusion of the cell membranes (usually with polyethylene glycol), the contents of the bacteria are delivered into the cytoplasm of the mammalian cells and the plasmid DNA is transferred to the

nucleus. Protoplast fusion is not as efficient as transfection for many of the cell lines that are commonly used for transient expression assays, but it is useful for cell lines in which endocytosis of DNA occurs inefficiently. Protoplast fusion frequently yields multiple copies of the plasmid DNA tandomly integrated into the host chromosome.

The application of brief, high-voltage electric pulses to a variety of mammalian and plant cells leads to the formation of nanometer-sized pores in the plasma membrane. DNA is taken directly into the cell cytoplasm either through these pores or as a consequence of the redistribution of membrane components that accompanies closure of the pores. Electroporation can be extremely efficient and can be used both for transient expression of clones genes and for establishment of cell lines that carry integrated copies of the gene of interest. Electroporation, in contrast to calcium phosphate-mediated transfection and protoplast fusion, frequently gives rise to cell lines that carry one, or at most a few, integrated copies of the foreign DNA.

Liposome transfection involves encapsulation of DNA and RNA within liposomes, followed by fusion of the liposomes with the cell membrane. In addition, DNA that is coated with a synthetic cationic lipid can be introduced into cells by fusion.

Direct microinjection of a DNA molecule into nuclei has the advantage of not exposing DNA to cellular compartments such as low-pH endosomes. Microinjection is therefore used primarily as a method to establish lines of cells that carry integrated copies of the DNA of interest.

F. Regulatory Functions of Polypeptides.

Typically, a transcription factor comprises a binding domain that binds to DNA and a regulatory domain that controls or regulates transcription. Where a regulatory domain activates transcription, that regulatory domain is designated an activation domain. Where a regulatory domain represses transcription, that regulatory domain is desginated an repression domain.

Egr-1 is one of only a small number of factors that contain modular domains capable of regulating transcription both positively and negatively.

Other examples include Krüppel [Zuo, *et al.* 1991], YY1/NF-E1/δ [Hahn 1992] and the immediate early factors Fos and Jun [Abate, *et al.* 1991]. The ability to work as either an activator or a repressor may be common to immediate early transcription factors to allow for versatility of effector functions. Post-translational modifications or interactions with cell-type specific factors may enable these complex transcription factors to function as either repressors or activators of transcription. Thus, Egr-1 may function differently in the diverse contexts in which it is expressed --immediately in response to growth stimuli in all cell types and with different kinetics and presumably target specificity in response to differentiation cues in some cell lineages.

1. Activation of transcription.

In an alternate embodiment of the invention, transcription on a polynucleotide is activated by a process comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes a polypeptide comprising a functional domain that activates transcription on a polynucleotide; and (2) maintaining that cell under physiological conditions sufficient to activate transcription.

Activation domains, and more recently repression domains, have been demonstrated to function as independent, modular components of transcription factors. Activation domains are not typified by a single consensus sequence but instead fall into several discrete classes: for example, acidic domains in GAL4 [Ma, *et al.* 1987], GCN4 [Hope, *et al.* 1986], VP16 [Sadowski, *et al.* 1988], and GATA-1 [Martin, *et al.* 1990]; glutamine-rich stretches in Sp1 [Courey, *et al.* 1988] and Oct-2/OTF2 [Müller-Immerglück, *et al.* 1990; Gerster, *et al.* 1990]; proline-rich sequences in CTF/NF-1 [Mermod, *et al.* 1989]; and serine/threonine-rich regions in Pit-1/GHF-1 [Theill, *et al.* 1989] all function to activate transcription. The activation domains of *Fos* and *Jun* are rich in both acidic and proline residues [Abate, *et al.* 1991; Bohmann, *et al.* 1989] and for other activators like the CCAAT/enhancer-binding protein C/EBP [Friedman, *et al.* 1990] no evident sequence motif has emerged. Fusions of the GAL4 DNA binding

domain and residues 1-281 of Egr-1 activate transcription some 100-fold. This N-terminal domain (***SEQ ID NO: 3***) is 30% serine-/threonine-/tyrosine-rich over a span of ~180 residues (aa 60 to 240) and includes several tracts of 5-7 consecutive serine or threonine residues. The large size of this activation domain may contribute to its potency relative to the smaller, previously described serine-/threonine-rich activator Pit-1 [Theill, *et al.* 1989]. Moreover, this transactivation domain is impervious to mutation in that substantial deletions in the extensive N-terminal domain do not impair transcriptional activity. It has been suggested that serine/threonine-rich domains may be phosphorylated and in this way function as acidic activators [Ptashne 1988; Hunter, *et al.* 1992]; in this regard, it is interesting to note that Egr-1 is phosphorylated [Christy, *et al.* 1988; Day, *et al.* 1990; and Waters, *et al.* 1990]. A second, weaker activation domain mapped to the C-terminus of Egr-1, which has octapeptide repeats reminiscent of the phosphorylated Tyr Ser Pro Thr Ser Pro Ser (***SEQ ID NO: 40***) reiterations in the carboxy-terminal domain of the RNA polymerase II large subunit [Corden 1990].

2. Repression of transcription.

In another embodiment of the invention, transcription on a polynucleotide is repressed by a process comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes a polypeptide comprising a functional domain that represses transcription on a polynucleotide; and (2) maintaining that cell under physiological conditions sufficient to repress transcription.

Where the regulatory domain of a transcription factor has a negative effect on transcription by the polynucleotide to which it is bound, then the transcription factor, or regulatory polypeptide, possesses a functional domain that can be characterized as repressing transcription. The novel repression domain of Egr-1 represents an extremely compact function of thirty-four amino acids that has been highly evolutionarily conserved through zebrafish, the lowest vertebrate in which a homologue of Egr-1 has been identified. The primary sequence of the Egr-1 repression domain, illustrated

in FIG. 7 and set out in ***SEQ ID NO. 5***, is not alanine- or glycine-rich as is the case for Krüppel, and suggested to be true for SCIP and YY1, and as such represents a transcriptional motif distinct from those observed previously.

Several models have been proposed as mechanisms of repression [Levine, *et al.* 1989]. Repressor proteins might act through competitive binding, either at the transcription start site or at the binding site of an upstream activating protein. Alternatively, a repressor might inhibit the activity of an activator or a component of the basal transcription apparatus without affecting, binding, by direct protein-protein interactions. By a third DNA binding-independent mechanism, termed "squelching," repression results from the titration of limiting factors essential for activation [Levine, *et al.* 1989]. Repression by GAL4-Egr-1 fusions is unlikely to be a result of squelching since the effect is DNA binding site-dependent. The mechanism may involve an interaction with a component of the basal transcription machinery since GAL4-Egr-1 (281 - 314) represses minimal promoter constructs. It is unlikely that repression occurs through displacement of basal factors by competition for binding sites because neither the GALA DNA-binding domain alone nor GAL4-Egr-1 (420-533) (data not shown) can mimic the repression seen with GAL4-Egr-1(281-314).

3. <u>Nuclear localization</u>.

In yet another embodiment of the present invention, nuclear localization in the expression of polypeptides is achieved by a process comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes a polypeptide comprising a functional domain that acts as a nuclear localization signal; and (2) maintaining that cell under physiological conditions sufficient to activate transcription.

Nuclear localization signals (NLS) are generally short stretches of 8 - 10 amino acids characterized by basic residues as well as proline. NLS sequences are retained in the mature protein, may be found at any position as long as it is exposed on the protein surface, and can be present in multiple copies. Proteins enter the nucleus through nuclear pores by a

two-step process: the first step is a rapid, signal-dependent binding to the nuclear pore periphery while the second step is a slower, ATP-and temperature-dependent translocation across the pore [Garcia-Bustos, *et al.* 1991; Silver 1991].

In Egr-1, basic residues cluster only in the finger domain and adjacent sequences suggesting that the karyophilic signal of Egr-1 resides here (***SEQ ID NOS: 6 and 7***; see FIG. 7). The basic region immediately 5' of the finger domain in combination with finger 2 or 3 is sufficient to target the bacterial protein β-galactosidase to the nucleus. This 5' basic stretch is conserved in other members of the EGR family (EGR2 and EGR3) which have shared DNA-binding domains but generally diverge outside this legion [Sukhat[illegible] 1990]. This is in agreement with suggestions that the C-terminus of Egr-1 is required for nuclear localization [Day *et al.*]. Precedents for the incorporation of nuclear targeting signals within a DNA-binding domain include *fos* [Tratner, *et al.* 1991]; the progesterone receptor, in which the second finger but not the first functions as an NLS [Guiochon-Mantel, *et al.* 1991]; GAL4 [Silver, *et al.* 1984]; and the homeo-domain proteins $\alpha 2$ and Pit-1/GHF-1 [Hall, *et al.* 1990; Theill, *et al.* 1989]. If nuclear localization signals and Cys_2His_2 finger domains -- both typified by basic residues-- have co-evolved, NLS sequences may generally be found adjacent to or integrated within zinc finger domains.

Other bipartite nuclear localization signals have been characterized in the polymerase basic protein 1 of influenza virus (PB1) [Nath, *et al.* 1990]; *Xenopus* protein N1 [Kleinschmidt, *et al.* 1988]; adenovirus DNA-binding protein (DBP) [Morin, *et al.* 1989]; and the yeast repressor $\alpha 2$ which has two nonhomologous signals, a basic NLS found at the N-terminus as well as a signal located in the homeodomain [Hall, *et al.* 1984 and 1990]. Because each $\alpha 2$ signal gives a different phenotype individually, [Hall *et al.*] sug[illegible] that these nonhomologous signals mediate separate steps in nuclear accumulation. The peripheral nuclear staining seen with $\alpha 2$ mutants with only the N-terminal NLS intact may reveal a signal for binding to but not translocation across the nuclear pore [Hall, *et al.* 1990]. This may be the

case for several Egr-1-β-galactosidase mutants containing the 5' basic stretch (but neither finger 2 nor 3 intact) which ring the nucleus and may be accumulating at nuclear pores.

4. Binding.

In another embodiment of the invention, binding to a polynucleotide is achieved by a process comprising: (1) transfecting a cell with an expression vector comprising a polynucleotide that encodes a polypeptide comprising a functional domain that binds to a polynucleotide; and (2) maintaining that cell under physiological conditions sufficient to repress transcription.

Gel mobility shift assays with extracts from HeLa cells transiently transfected with the series of internal deletion derivatives show that only amino acids 331-419 (*SEQ ID NO: 8*) of Egr-1 (encoding the three zinc fingers) are required for specific DNA-binding. (See FIG. 7.) Deletion N-terminal of the zinc finger domain (eight amino acids 5' of the first cysteine) has no effect on DNA-binding. The deletion C-terminal of the zinc fingers (four amino acids 3' of the last histidine) may slightly impair DNA-binding. Western analysis with polyclonal anti Egr-1 antisera indicates that the loss of DNA-binding activity with deletions within the zinc finger domain is not due to a reduction in protein expression.

G. Pharmaceutical Compositions.

An alternative embodiment of the present invention contemplates a pharmaceutical composition comprising a mammalian early growth regulatory polypeptide and a physiologically acceptable carrier. Yet another embodiment provides a pharmaceutical composition comprising a polypeptide that comprises a functional domain that activates transcription, and a physiologically acceptable carrier. Still another embodiment of the present invention provides a pharmaceutical composition comprising a polypeptide that comprises a functional domain that represses transcription and a physiologically acceptable carrier.

An embodiment of the present invention also provides a pharmaceutical composition comprising a polypeptide that comprises a functional domain that functions as a nuclear localization signal, and a physiologically

acceptable carrier. Alternatively, the present invention provides a pharmaceutical composition comprising a polypeptide that comprises a functional domain that binds to a polynucleotide, and a physiologically acceptable carrier.

In another embodiment, the present invention provides a pharmaceutical composition comprising a polynucleotide that encodes a mammalian early growth regulatory polypeptide and a physiologically acceptable carrier. Another embodiment provides a pharmaceutical composition comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that activates transcription, and a physiologically acceptable carrier. Alternatively, the present invention contemplates a pharmaceutical composition comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that represses transcription, and a physiologically acceptable carrier.

In yet another embodiment, the present invention contemplates a pharmaceutical composition comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that acts as a nuclear localization signal, and a physiologically acceptable carrier. In still another embodiment, the present invention provides a pharmaceutical composition comprising a polynucleotide that encodes a polypeptide that comprises a functional domain that binds to a polynucleotide, and a physiologically acceptable carrier.

A composition of the present invention is typically administered parenterally in dosage unit formulations containing standard, well-known nontoxic physiologically acceptable carriers, adjuvants, and vehicles as desired. The term parenteral as used herein includes intravenous, intramuscular, intraarterial injection, or infusion techniques.

Injectable preparations, for example sterile injectable aqueous or oleaginous suspensions, are formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension

in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol.

Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Preferred carriers include neutral saline solutions buffered with phosphate, lactate, Tris, and the like. Of course, one purifies the vector sufficiently to render it essentially free of undesirable contaminant, such as defective interfering adenovirus particles or endotoxins and other pyrogens such that it does not cause any untoward reactions in the individual receiving the vector construct. A preferred means of purifying the vector involves the use of buoyant density gradients, such as cesium chloride gradient centrifugation.

A carrier can also be a liposome. Means for using liposomes as delivery vehicles are well known in the art [*See, e.g.*, Gabizon *et al.*, 1990; Ferruti *et al.*, 1986; and Ranade, V.V., 1989].

A transfected cell can also serve as a carrier. By way of example, a liver cell can be removed from an organism, transfected with a polynucleotide of the present invention using methods set forth above and then the transfected cell returned to the organism (e.g. injected intravascularly).

H. Immunoreactive Antibodies.

The invention also contemplates an antibody specifically immunoreactive with a mammalian early growth regulatory protein or a polynucleotide encoding a mammalian early growth regulatory polypeptide. (see Example 4). Preferably, the antibody is a monoclonal antibody. More preferably, the antibody specifically immunoreacts with the amino acid residue sequence: His Leu Arg Gln ys Asp Lys Lys Ala Asp Lys Ser Lys (*SEQ ID NO: 38*), or with the amino acid residue sequence: Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His Thr Lys Ile (*SEQ ID NO: 39*).

Means for preparing and characterizing antibodies are well known in the art (*See, e.g., Antibodies "A Laboratory Manual,* E. Howell and D. Lane, Cold Spring Harbor Laboratory, 1988).

Briefly, a polyclonal antibody is prepared by immunizing an animal with an immunogen comprising a polypeptide or polynucleotide of the present invention, and collecting antisera from that immunized animal. A wide range of animal species can be used for the production of antisera. Typically an animal used for production of anti-antisera is a rabbit, a mouse, a rat, a hamster or a guinea pig. Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

As is well known in the art, a given polypeptide or polynucleotide may vary in its immunogenicity. It is often necessary therefore to couple the immunogen (e.g., a polypeptide or polynucleotide) of the present invention) with a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers.

Means for conjugating a polypeptide or a polynucleotide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimyde and bis-biazotized benzidine.

As is also well known in the art, immunogencity to a particular immunogen can be enhanced by the use of non-specific stimulators of the immune response known as adjuvants. Exemplary and preferred adjuvants include complete Freund's adjuvant, incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

The amount of immunogen used of the production of polyclonal antibodies varies *inter alia,* upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal. The production of polyclonal antibodies is monitored by

sampling blood of the immunized animal at various points following immunization. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored.

A monoclonal antibody of the present invention can be readily prepared through use of well-known techniques such as those exemplified in U.S. Pat. No 4,196,265, herein incorporated by reference.

Typically, a technique involves first immunizing a suitable animal with a selected antigen (e.g., a polypeptide or polynucleotide of the present invention) in a manner sufficient to provide an immune response. Rodents such as mice and rats are preferred animals. Spleen cells from the immunized animal are then fused with cells of an immortal myeloma cell. Where the immunized animal is a mouse, a preferred myeloma cell is a murine NS-1 myeloma cell.

The fused spleen/myeloma cells are cultured in a selective medium to select fused spleen/myeloma cells from the parental cells. Fused cells are separated from the mixture of non-fused parental cells, for example, by the addition of agents that block the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides. Where azaserine is used, the media is supplemented with hypoxanthine.

This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants for reactivity with an antigen-polypeptides. The selected clones can then be propagated indefinitely to provide the monoclonal antibody.

By way of specific example, to produce an antibody of the present invention, mice are injected intraperitoneally with between about 1-200 μg of an antigen comprising a polypeptide of the present invention. B

lymphocyte cells are stimulated to grow by injecting the antigen in association with an adjuvant such as complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*). At some time (e.g., at least two weeks) after the first injection, mice are boosted by injection with a second dose of the antigen mixed with incomplete Freund's adjuvant.

A few weeks after the second injection, mice are tail bled and the sera titered by immunoprecipitation against radiolabeled antigen. Preferably, the process of boosting and titering is repeated until a suitable titer is achieved. The spleen of the mouse with the highest titer is removed and the spleen lymphocytes are obtained by homogenizing the spleen with a syringe. Typically, a spleen from an immunized mouse contains approximately 5×10^7 to 2×10^8 lymphocytes.

Mutant lymphocyte cells known as myeloma cells are obtained from laboratory animals in which such cells have been induced to grow by a variety of well-known methods. Myeloma cells lack the salvage pathway of nucleotide biosynthesis. Because myeloma cells are tumor cells, they can be propagated indefinitely in tissue culture, and are thus denominated immortal. Numerous cultured cell lines of myeloma cells from mice and rats, such as murine NS-1 myeloma cells, have been established.

Myeloma cells are combined under conditions appropriate to foster fusion with the normal antibody-producing cells from the spleen of the mouse or rat injected with the antigen/polypeptide of the present invention. Fusion conditions include, for example, the presence of polyethylene glycol. The resulting fused cells are *hybridoma* cells. Like myeloma cells, hybridoma cells grow indefinitely in culture.

Hybridoma cells are separated from unfused myeloma cells by culturing in a selection medium such as HAT media (hypoxanthine, aminopterin, thymidine). Unfused myeloma cells lack the enzymes necessary to synthesize nucleotides from the salvage pathway because they are killed in the presence of aminopterin, methotrexate, or azaserine. Unfused lymphocytes also do not continue to grow in tissue culture. Thus,

only cells that have successfully fused (hybridoma cells) can grow in the selection media.

Each of the surviving hybridoma cells produces a single antibody. These cells are then screened for the production of the specific antibody immunoreactive with an antigen/polypeptide of the present invention. . Single cell hybridomas are isolated by limiting dilutions of the hybridomas. The hybridomas are serially diluted many times and, after the dilutions are allowed to grow, the supernatant is tested for the presence of the monoclonal antibody. The clones producing that antibody are then cultured in large amounts to produce an antibody of the present invention in convenient quantity.

By use of a monoclonal antibody of the present invention, specific polypeptides an polynucleotides of the invention can be recognized as antigens, and thus identified. Once identified, those polypeptides and polynucleotides can be isolated and purified by techniques such as <u>antibody-affinity chromatography</u>. In antibody-affinity chromatography, a monoclonal antibody is bound to a solid substrate and exposed to a solution containing the desired antigen. The antigen is removed from the solution through an immunospecific reaction with the bound antibody. The polypeptide or polynucleotide is then easily removed from the substrate and purified.

I. <u>Diagnostic Assay Kits</u>

In another aspect, the present invention contemplates diagnostic assay kits for detecting the presence of mammalian early growth regulatory polypeptides in biological samples, where the kits comprise a first container containing a first antibody capable of immunoreacting with mammalian early growth regulatory polypeptides, with the first antibody present in an amount sufficient to perform at least one assay. Preferably, the assay kits of the invention further comprise a second container containing a second antibody that immunoreacts with the first antibody. More preferably, the antibodies used in the assay kits of the present invention are monoclonal antibodies. Even more preferably, the first antibody is affixed to a solid support. More

preferably still, the first and second antibodies comprise an indicator, and, preferably, the indicator is a radioactive label or an enzyme.

In an alternative aspect, the present invention provides diagnostic assay kits for detecting the presence, in biological samples, of polynucleotides that encode mammalian early growth regulatory polypeptides, the kits comprising a first container that contains DNA probe molecules that are complementary to a sequence of from about 15 to about 40 contiguous nucleotide bases of ***SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14***, or of any of their complements, wherein the DNA probe molecules are capable of hybridizing to polynucleotides that encode mammalian early growth regulatory polypeptides.

In another embodiment, the present invention contemplates diagnostic assay kits for detecting the presence, in a biological sample, of antibodies immunoreactive with mammalian early growth regulatory polypeptides, the kits comprising a first container containing mamamlian early growth regulatory polypeptides that immunoreact with the antibodies, with the polypeptides present in an amount sufficient to perform at least one assay.

J. <u>Detection Methods</u>.

In still another embodiment, the present invention contemplates a process, as illustrated in Example 8, of detecting a mammalian early growth regulatory polypeptide comprising immunoreacting the polypeptide with an antibody that is specifically immunoreactive to form a conjugate, and detecting the conjugate.

The invention also contemplates a process of detecting a messenger RNA transcript that encodes a mammalian early growth regulatory polypeptide, which process comprises hybridizing RNA with a polynuc[illegible] sequence that encodes a mammalian early growth regulatory polypeptide. Preferably, the present invention provides a process of detecting a DNA molecule that encodes for a mammalian early growth regulatory polypeptide,

which process comprises hybridizing a sample of DNA with a polynucleotide that encodes a mammalian early growth regulatory polypeptide.

In another embodiment, the invention provides a method of detecting a disease genetically linked to a mammalian Egr gene comprising the step of quantitating mammalian early growth regulatory polynucleotide sequences encoding a mammalian early growth regulatory polypeptide.

1. Probes and Primers.

In another aspect, polynucleotide sequence information provided by the present invention allows for the preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to gene sequences of the selected polynucleotides disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared based on consideration of a selected nucleotide sequence, e.g., a sequence such as that shown in Figure 1 and given by ***SEQ ID NO: 1***. The ability of such nucleic acid probes to specifically hybridize to a polynucleotide encoding a polypeptide that activates transcription on a polynucleotide lends them particular utility in a variety of embodiments. Most importantly, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample (see Example 8).

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of a gene or polynucleotide that encodes a polypeptide containing specific regulatory domains from mammalian cells using PCR technology. (See Table 4, Example 9, *infra*.)

To provide certain of the advantages in accordance with the present invention, a preferred nucleic acid sequence employed for hybridization studies or assays includes sequences that are complementary to at least a 10- to 50-nucleotide long stretch of a polynucleotide that encodes a polypeptide that activates transcription on a polynucleotide, such as that corresponding to ***SEQ ID NO: 9 or SEQ ID NO: 10***. A size of at least 10 nucleotides in length helps to ensure that the fragment will be of sufficient

length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 10 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 20 nucleotides, 30 to 50 nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR technology of U.S. Patent 4,603,102, herein incorporated by reference, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction sites.

Accordingly, a nucleotide sequence of the invention can be used for its ability to selectively form duplex molecules with complementary stretches of the gene. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degree of selectivity of the probe toward the target sequence. For applications requiring a high degree of selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids. For example, one will select relatively low salt and\or high temperature conditions, such as provided by 0.02 M-0.15 M NaCl at temperatures of 50° C to 70° C. Those conditions are particularly selective, and tolerate little, if any, mismatch between the probe and the template or target strand.

Of course, for some applications, for example where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template, or where one seeks to isolate sequences coding polypeptides that activate transcription on a polynucleotide from other cells, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as 0.15 M - 0.9 M salt, at temperatures ranging from 20°C to 55°C. Cross-hybridizing

species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. Generally, it is appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it is advantageous to employ a polynucleotide of the present invention in combination with an appropriate label for detecting hybrid formation. A wide variety of appropriate labels are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal.

In general, it is envisioned that a hybridization probe described herein is useful both as a reagent in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions depend as is well known in the art on the particular circumstances and criteria required (e.g., on the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe). Following washing of the matrix to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label.

The following examples illustrate particular embodiments of the present invention and are not limiting of the specification and claims in any way.

EXAMPLE 1: Preparation and Structural Analysis of cDNA for Mouse Egr-1.

Isolation of DNA encoding a mammalian early growth regulatory protein including one or more histidine-cysteine zinc finger amino acid sequences was performed substantially according to the procedures des-

cribed in [Sukhatme *et al.* (1987)], the disclosures of which are specifically incorporated by reference herein.

Balb/c 3T3 cells (clone A31) from the American Type Culture Collection were grown to confluence in Dulbecco's Modified Eagle's medium (DME) supplemented with 10% fetal calf serum (FCS). The cells were rendered quiescent by reduction of the serum concentration to 0.75% for 48 hours. To induce the cells from quiescence into growth phase G, the medium was changed to 20% FCS with cycloheximide added to a final concentration of 10 μg/ml.

RNA was extracted from Balb/c 3T3 cells harvested three hours after induction of quiescent cells by 20% FCS and 10 μg/ml cycloheximide. A λgt10 cDNA library was constructed from this mRNA according to the procedures of [Glover 1985]. This library was screened differentially with single stranded cDNA prepared from quiescent cells and from cells exposed to serum and cycloheximide for 3 hours. These ^{32}P-labeled cDNA probes were prepared from poly A^{+} RNA as described in [St. John, *et al.* 1979], except that 100 μCi of ^{32}P-dCTP (>3000 Ci/mmol), 0.02 mM cold dCTP and 2-5 μg of poly A^{+} RNA was used in each reaction. The mean size of the reverse transcribed probes, as assessed by alkaline agarose gel electrophoresis and subsequent autoradiography, was about 700 bases. Replica filter lifts (GeneScreenPlus, NEN-DuPont) were prepared essentially as described by [Benton *et al.* 1977], and approximately 3 x 10^{6} cpm of ^{32}P-cDNA were used per filter (90 mm diameter). Hybridizations were carried out at 65°C in 1% SDS, 10% dextran sulfate, and 1 M NaCl for a period of 16 hours. The filters were washed twice for twenty minutes each time, first at room temperature in 2 x SSC [Maniatis *et al.* 1982], then at 65°C in 2 x SSC, 1% NaDodSO$_4$ and finally at 65°C in 0.2 x SSC. Autoradiograms were prepared by exposing the blots for 18 hours at -[illegible] with an intensifying screen.

A total of 10,000 cDNA clones from the Balb/c 3T3 λgt10 library were differentially screened. Seventy-eight clones were found to hybridize preferentially to single-stranded cDNA from fibroblasts stimulated for 3

hours with 20% FCS and cycloheximide as compared to single-stranded cDNA from quiescent cells. Inserts from these clones were cross-hybridized to each other, resulting in the sorting of forty clones into 7 cDNA families one of which was identified as *c-fos*. Another cDNA clone, referred to as OC68, contained a 2.2 kb insert and was characterized further. This insert was subcloned into the EcoRI site of pUC13, and probes were generated for Northern blot analysis either from the insert or the corresponding pUC plasmid. Two RsaI digestion fragments, derived from the 5' end of clone OC68 and each comprising approximately 130 base pairs, were labeled and employed to re-screen the above-described λgt10 cDNA library, resulting in the recovery of a 3.1 kb clone, designated OC3.1. This clone was sequenced according to the method of [Sanger *et al.* 1977]. The 3086 base pair sequence obtained is set forth in FIG. 1 (***SEQ ID NO: 15***), along with the deduced sequence of 533 amino acid residues for the protein encoded, designated mouse "Egr-1" (***SEQ ID NO: 1***).

The deduced amino acid sequence shows a single long open reading frame with a stop codon (TAA) at position 1858. The most 5', in-frame, ATG, at position 259, is flanked by sequences that fulfill the Kozak criterion ($^{A}_{G}$ NN(ATG)G) [Kozak 1987]. The sequence region upstream of this ATG is highly GC-rich and results in an absence of in-frame stop codons. The 3' untranslated region (UT) contains two "AT" rich regions (nucleotides 2550-2630 and 2930-2970). Similar sequences are found in the 3' UT regions of several lymphokine and proto-oncogene mRNAs, including granulocyte macrophage colony stimulating factor (GM-CSF), interleukin 1, interleukin 2, interleukin 3 (IL-3), α, β, and γ interferons, and c-fos, c-myc, and c-myb [Shaw *et al.* 1986]. These sequences may mediate selective mRNA degradation. The presence in the mouse Egr-1 transcript of such regions is consistent with its short message half-life. Potential polyadenylation signals (AATAAA) are located at nucleotide positions 1865 and 3066, as well as at position 3053 (AATTAA) [Wickens *et al.* 1984].

The deduced amino acid sequence predicts a polypeptide of 533 amino acids with a molecular weight of 56,596. Based on structural

considerations, namely a central region containing zinc fingers, the Egr-1 protein can be divided into three domains. The N-terminal portion (amino acid residues 2 to 331) is rich in proline (14.2%) and serine (16%) residues with 7.9% alanines and 7.9% threonines. The C-terminal region (residues 417 to 533) also contains a very high proportion of prolines and serines (15.4 and 26.5%, respectively) and 10.3% alanines and 11.1% threonines. The large number of proline residues leads to a secondary structure that probably lacks α-helices. The central portion of the Egr-1 protein consists of three tandem repeat units of 28-30 amino acids, with the first unit starting at position 332. Each unit conforms almost exactly to the consensus sequence Thr Gly Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa His Xaa Xaa Xaa His ($X_3{}^{Y}_{F}$ $XCX_{2-4}CX_3FX_5LX_2HX_3H$) (see FIG. 3) (*SEQ ID NO: 16*), diagnostic of DNA binding zinc fingers [Berg 1986; Brown *et al.* 1986; and Brown *et al.* 1985]. Furthermore, the Egr-1 fingers are connected by "H-C links" (TGE^{R}_{K} P^{F}_{Y} X) (*SEQ ID NO: 41*) [Schuh *et al.* 1986] found in the Xenopus TFIIIA gene (between fingers 1, 2, and 3), in the Drosophila Kruppel gap gene [Rosenberg *et al.* 1986], and in genes from mouse and Xenopus that cross-hybridize to the Kruppel (Kr) finger domains: mkr1, mkr2 [Chowdhury *et al.* 1987], and Xfin [Altaba *et al.* 1987]. The sequence similarity amongst the Egr-1 fingers is 50-70%, whereas the sequence similarity between any of the Egr-1 fingers and those present in TFIIIA, Kruppel, mkr1, mkr2 or Xfin is 35-40%. Outside of the finger domains, it is noteworthy that the Egr-1 and Kr proteins each contain a very high proportion of Pro, Ala, and Ser residues [Schuh *et al.* 1986]. However, there is no sequence similarity in these regions. Thus, Egr-1 and Kr are not homologous genes nor is Egr-1 related to mkr1, mkr2, Xfin, or TFIIIA. The Kr gene contains thirteen copies of the hexanucleotide (ACAAAA), or its complementary sequence, eight of which are located within 180 bp downstream from the Kr TATA box and five are in the 3' UT region. These sequences may serve as targets for other DNA binding proteins or in Kr gene autoregulation. The Egr-1 cDNA also contains nine copies of the ACAAAA sequence or its complement.

Following the work described above, [Milbrandt 1988], reported the isolation and sequence of a nerve growth factor (NGF) inducible cDNA (NGFI-A) from the rat pheochromocytoma PC12 line. A comparison of the deduced amino acid sequence of NGFI-A to that of mouse Egr-1 of FIG. 1 reveals 98% sequence identity. Thus, mouse Egr-1 and rat NGFI-A are homologs. The putative initiation ATG chosen by Milbrandt corresponds to position 343 in the FIG. 1 cDNA sequence, and is 84 nucleotides (28 amino acid residues) downstream of the ATG therein designated for translation initiation. Both ATG's have a purine at position -3 and a G at position +1 and the designation represented in FIG. 1 of the more 5' ATG as the p[illegible]tive start codon is based on the experience of [Kozak 1987], even tho[illegible] the more 3' ATG is surrounded by the longer Kozak consensus sequence (CCG/ACCATGG). Translation of an *in vitro* generated RNA transcript, described <u>infra</u>, selects the more 5' ATG for initiation.

It is noteworthy that a major difference in the deduced sequences of mouse Egr-1 and rat NGFI-A resides in the sequence spanning residues 61 - 68 of Egr-1 and 33 - 43 of NGFI-A. The former includes the sequence Asn Ser Ser Ser Ser Thr Ser Ser (*SEQ ID NO: 42*) while the latter includes the sequence Asn Asn Ser Ser Ser Ser Ser Ser Ser Ser Ser (*SEQ ID NO: 43*), accounting for the 3 residue difference in length of the putative polypeptides which is not accounted for by the difference in designation of the transcript initiation signal.

EXAMPLE 2: <u>Human Chromosome Gene Mapping</u>.

To determine the human chromosomal localization of the gene corresponding to mouse Egr-1, the OC3.1 and OC19t cDNA clones were hybridized to a panel of rodent x human somatic cell hybrids. Southern blot analysis of the hybrid panel showed concordance between the presence [illegible] Egr-1 sequences and human chromosome 5. *In situ* hybridization to no[illegible] human metaphase chromosomes resulted in specific labeling only of chromosome 5, with the largest cluster of grains at 5q23-31. Specific labeling of these bands was also observed in hybridizations using an Egr-1 probe which does not contain finger sequences.

This localization is interesting in light of the non-random deletions [del(5q)] in human myeloid disorders (acute myelogenous leukemia) (AML), and myelodysplastic syndromes, that involve this chromosomal region. [Le Beau 1986; Dewald *et al.* 1985; and Van den Berghe *et al.* 1985]. Fifty percent of patients with therapy-related AML show chromosome 5 abnormalities (interstitial deletions or monosomy) and cytogenetic analysis of the deletions has revealed that one segment, consisting of bands 5q23-31, is absent in the malignant cells of all patients who have aberrations of chromosome 5. These data suggest that loss of a critical DNA sequence leading to hemizygosity (or homozygosity) of a recessive allele may play an important role in the pathogenesis of these disorders, a mechanism substantiated for retinoblastoma. Although genes for a number of growth factors and receptors (IL-3, GM-CSF, β2-adrenergic receptor, endothelial cell growth factor, CSF-1, c-fms, pDGF receptor) are clustered in or near this region, Egr-1 (by virtue of its zinc fingers) is the only member of this group with potential transcriptional regulatory activity. It is therefore possible that its absence could lead to deregulated cell growth.

EXAMPLE 3: In Vitro Expression of Mouse Egr-1 cDNA.

A 2.1 kb ApaI/ApaI fragment (comprising nucleotides 120-2224 of FIG. 1 was isolated from the OC3.1 DNA clone. This fragment includes the translation start (ATG) codon at nucleotide position 259 designated in FIG. 1. The fragment was blunt-ended with T4 DNA polymerase and cloned into the Bluescript vector KS M13(+) containing a T3/T7 bacteriophage promoter. The (T3) sense transcript was generated and *in vitro* translated in a standard rabbit reticulocyte lysate system (Promega Biotec, Madison, WI. 53711) including ^{35}S methionine as a radiolabel. An analogous *in vitro* transcription system was developed using a Bg1II/Bg1II fragment of OC3.1 (including nucleotides 301-1958 and not including the translation start designated in 1A. The T7 sense transcript was employed in the translation system. Differential characterization of translation products by autoradiographic SDS PAGE indicated that the ATG at nucleotide position

259 is preferred as a translation start codon when all potential start sites are present.

EXAMPLE 4: Preparation of Antibodies.

A first synthetic peptide based on the sequence of amino acid residues 416-427 of mouse Egr-1 was prepared and provided with a carboxy terminal cysteine residue. The peptide, His Leu Arg Gln ys Asp Lys Lys Ala Asp Lys Ser Lys (*SEQ ID NO: 38*), was coupled to KLH and employed to immunize New Zealand white rabbits. Animals were initially immunized with 100 μg of the immunogen in Freund's Complete Adjuvant and every two weeks were boosted with 100 μg of immunogen with Freund's Incomplete Adjuvant. Sera, designated VPS10, were isolated after 68 days and displayed an antibody titer of 1:12,800 based on reactivity with the antigen used to prepare the antisera.

A second synthetic peptide, based on residues 399 to 415 of mouse Egr-1, was prepared. The peptide, Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His Thr Lys Ile (*SEQ ID NO: 39*), was coupled to KLH and used to immunize rabbits as above, resulting in the production of antisera (designated VPS2) with a titer of 1:400.

EXAMPLE 5: Isolation of Genomic Mouse Egr-1 Clone and Characterization of Regulatory Regions.

A mouse Balb/c 3T3 genomic library was prepared in a Stratagene (La Jolla, California) vector, λFIX, according to the manufacturer's instructions and probed using 1% SDS, 1 M NaCl, and 10% dextran sulfate at 65°C with stringent final wash in 0.2 x SSC at 65°C with a 2.1 kb ApaI/ApaI fragment and a 3.1 kb Eco RI/EcoRI fragment derived from digestion of pUC13 including the mouse Egr-1 clone OC3.1. One positive clone, from approximately 300,000 screened, was designated mgEgr-1.1, and also hybridized to the extreme 5'-end 120 bp EcoRIApaI fragment from plasmid OC3.1.

A 2.4 kb Pvu-II-PvuII fragment and a 6.6 kb XbaI-XbaI fragment derived from the mgEgr-1.1 clone were subcloned into the SmaI and XbaI sites of pUC13 and pUC18 respectively, and the resulting plasmids

(designated as p2.4 and p6.6) were used for restriction mapping analysis of transcription initiation sites and for nucleotide sequencing. Listed in Table 3

TABLE 3

LOCATION AND IDENTIFICATION OF POTENTIAL REGULATORY ELEMENTS

Element Location	Sequence	
TATA	AAATA	-26 to -22
CCAAT	CCAAT	-337 to -333
Serum Response Element Consensus		
GATGTCCATATTAGGACATC (SEQ ID NO: 44)	TCCTTCCATATTAGGGCTTC (SEQ ID NO: 47)	-110 to -91
CC TA AT GG (SEQ ID NO: 45)	GTGGCCC-AATATGGCCCTG (SEQ ID NO: 48)	-342 to -324
G C (SEQ ID NO: 46)	CAGCGCCTTATATGGAGTGG (SEQ ID NO: 49)	-358 to -339
	ACAGACCTTATTTGGGCAGC (SEQ ID NO: 50)	-374 to -355
	AAACGCCATATAAGGAGCAG (SEQ ID NO: 51)	-412 to -393
TPA Responsive Element (AP1 binding site) Consensus		
C C TGACT A G A	CTGACTCG	-610 to -603
	CTGACTGG	-867 to -860
Sp1 binding site	GGGCGG	-285 to -280
	GGGCGG	-649 to -644
	CCGCCC	-700 to -695
	GGGCGG	-719 to -714
cAMP Response Element Consensus		
TGACGTCA	TCACGTCA	-138 to -131
	TGACGGCT	-631 to -624

are possible regulatory elements identified in the 5' flanking sequence of mgEgr-1.1. A putative TATA motif (AAATA) is located 26 nucleotides upstream of the transcription start site. A "CCAAT" type sequence starts at nucleotide -337. Five different regions, each 10 nucleotides in length, located at -110, -342, -358, -374, and -412, are nearly identical to the inner core of the c-fos serum response element [Treisman 1986].

Each has a 5-6 nucleotide AT rich stretch and is surrounded by the dinucleotide CC on the 5' side and GG on the other. Two potential TPA responsive elements [Lee, *et al* 1987 and Angel *et al* 1987] are located at nucleotides -610 and -867. Four consensus Sp1 [Briggs 1986]] binding sequences are at position -285, -649, -700 and -719. In addition, two sequences have been identified that might serve as cAMP response elements [Montimy *et al*. 1987] (-138 and -631).

To obtain the genomic sequence and the intron/exon gene structure, specific oligonucleotides (17-mers at positions 83, 122, 174, 200, 379, 543, 611, 659, 905, 920, 1000, 1200, 1400, 1600, 1800, 2100, 23 2650, 2825) of the OC3.1 cDNA sequence (see FIG. 1) were used as primers for double stranded sequencing of plasmids p2.4 and p6.6. Comparison of the Egr-1 genomic sequence to the Egr-1 cDNA sequence showed the Egr-1 gene consists of 2 exons and a single 700 bp intron (between nucleotide position 556 and 557 as numbered in FIG. 1). Both the 5' and 3' splice junction sequences (not shown) are in excellent agreement with the consensus boundary sequences. [Mount 1982].

EXAMPLE 6: Isolation and Characterization of Human EGR2 cDNA.

A human genomic placental library in the vector EMBL3, prepared by Dr. C. Westbrook of the University of Chicago according to procedures described in [Frischauff *et al.* 1983], and a human leukocyte cosmid library prepared according to procedures described were probed with the 2.1 kb ApaI fragment of OC3.1 (described in Example 5) using 1% SDS, 1 M NaCl and 10% dextrose sulfate at 50-55°C with a non-stringent final wash in 2 x SSC at 50-55°C. A single positive clone (designated HG6) was isolated from the first library and four clones (designated HG17, 18, 19 and 21, respectively) were isolated from the second library. A 6.6 kb SalI/EcoR fragment of clone HG6 was found to hybridize with a 332 base pair HpaII/HpaII fragment of the mouse Egr-1 gene, which letter fragment spans the putative zinc finger region. The 6.6 kb fragment, in turn, was employed to probe a cDNA library derived from human fibroblasts which have been

stimulated for three hours with 20% fetal calf serum in the presence of 10 μg/ml cyclohexamide.

About 10,000 clones were screened and the fifty positive clones obtained (designated "zap-1 through zap-50") are being subjected to nucleotide sequence analysis. Preliminary sequence analysis reveals that three clones, zap-2, zap-8, and zap-32, all encode the same transcript, namely a protein designated human EGR2, shown in FIG. 4. Preliminary analysis indicates approximately 92% homology between mouse Egr-1 and human EGR2 polypeptides in the zinc finger regions, but substantially less homology in the amino and carboxy terminal regions. Chromosome mapping studies, similar to those described in Example 2, indicate that human chromosome 10, at bands q21-22, constitutes a locus for the human EGR2 gene.

The plasmid zap-32, containing the full length human EGR2 clone, was used as a probe in Southern blot analysis on DNAs from 58 unrelated Caucasians. It was found that Hind III detects a simple two-allele polymorphism with bands at either 8.0 kb (A1) or 5.6 kb and 2.4 kb (A2). No constant bands were detected. The frequency of A1 was 0.90 and that of A2 was 0.10. No polymorphisms were detected for Apa I, BamH I, Ban II, Bg1 I, Bg1 II, BstE II, Dra I, EcoR I, EcoR V, Hinc II, Msp I, Pst I, Pvu II, Rsa I, Sac I, and Taq I in 10 unrelated individuals. Co-dominant segregation of the Hind III RFLP was observed in four large kindreds with a total of more than 350 individuals.

These data will be useful in gene linkage studies for mapping genes for certain genetic disorders. For example, the gene responsible for the dominantly inherited syndrome, multiple endocrine neoplasia, type 2A (MEN-2A) has been assigned by linkage to chromosome 10. [Simpson, *et al.* 1987.] Studies are currently underway to determine the linkage relationship between MEN-2A and EGR2 and are expected to be useful in cloning the MEN-2A gene as well as in serving as a diagnostic marker for the disease.

EXAMPLE 7: <u>Recombinant Expression of Fusion Proteins</u>.

A 322 base HpaII/HpaII fragment (comprising nucleotides 1231 - 1553) derived from the OC3.1 cloned DNA was treated with DNA polymerase to fill in the single stranded ends. This fragment was inserted in plasmid pEX3 (obtained from K. Stanley, European Molecular Biology Laboratory, Postfach 10.2209, 6900 Heidelberg, F.R.G.) digested with SmaI. [Stanley, K.K., *et al.* 1984.] This insertion placed the Egr-1 encoding DNA fragment in the same reading frame as plasmid DNA encoding cro-β-galactosidase, allowing for the expression of a fusion protein comprising the amino terminal residues of cro-β-galactosidase and 108 residues of Egr-1 amino acids 325 to 432. This cro-β-galactosidase/Egr-1 fusion plasmid, designated pFIG, was used to transform *E. coli* NF1.

Induced (42°C) and un-induced (30°C) cultured cell lysates from growth of the transformed NF1 cells were then analyzed by SDS-PAGE. Upon Coomassie stain analysis, only induced cell lysates included an approximately 108 kd product, indicating presence of the projected expression product. Western blot analysis, using the rabbit polyclonal anti-peptide antibody VPS10, raised against His Leu Arg Gln Lys Asp Lys Lys Ala Asp Lys Ser Lys (*SEQ ID NO: 38*), confirmed that the fusion protein product contained Egr sequences.

In a separate construction, a mouse Egr-1 insert, from plasmid OC3.1, was fused, in-frame, to a plasmid containing sequences from bovine growth hormone according to the methods described in [Slamon, *et al.* 1986]. The resultant plasmid, designated pV4, comprised a fusion protein containing a fusion gene coding for bovine growth hormone amino acids 1 to 192 and Egr-1 amino acids 2 to 533. This bGH/mouse Egr-1 DNA fusion plasmid, designated pV4, was expressed in <u>E. coli</u> and the resulting fusion protein, designated V4, was identified in Western blots by its reactivity with a bGH monoclonal antibody and its reactivity with VPS10 rabbit anti-Egr-1 peptide antiserum.

EXAMPLE 8: Determination of Egr Levels
in Human Tumor and Non-Tumor Tissue.

Using the mouse Egr-1 OC68 probe, Northern blot analyses were conducted to determine the levels of transcription of Egr protein encoding DNA in tumor versus surrounding normal tissue from resected human tumor specimens. The tumor samples were from lung (12), colon (7), colon mesastasis (1), bladder (1), rectal (1), giant cell (1), hepatoma (1), breast (1), MFH (malignant fibrous histiocytoma) (1), osteosarcoma (1) and rhabdomyosarcoma (1). In about 50% of these cases, there is markedly decreased (about three to ten-fold) expression of the Egr mRNA in tumor versus normal tissue. One implication of this finding is that Egr proteins of the invention may function as part of a negative regulatory pathway. In any event, it is clear that DNA sequences and antibodies of the invention are susceptible to use in differential diagnoses between tumorous and non-tumorous cell types.

EXAMPLE 9: Mutagenesis of the amino acid coding region of
Egr-1 cDNA in a cytomegalovirus expression vector.

In order to determine the functional domains of Egr-1, the 533 amino acid coding region of the murine Egr-1 cDNA was subjected to site-directed mutagenesis to create a series of in-frame internal deletions of 43 - 48 amino acids (see Figure 8). The approach taken was to define domains important for transcriptional activity (both repression and activation), DNA-binding, and nuclear localization by looking for loss of function with deletion derivatives of Egr-1.

Each of the Egr-1 deletion derivatives is expressed from the vector pCB6+, a modified version of pCB6, comprising EcoRI, NotI, and EcoRV sites. Vector pCB6+ contains the cytomegalovirus (CMV) early promoter/enhancer followed by a polylinker and human growth hormone polyadenylation signal as well as the gene for neomycin resistance expressed from the SV40 early promoter/ enhancer. The parent expression vector for all deletion derivatives, pCB.Egr-1, consists of the full-length murine Egr-1 cDNA cloned into the EcoRI site of pCB6+ and has been described previously (42). Site-directed mutagenesis was used to produce nine

in-frame internal deletions of 43-48 amino acids and one deletion of 24 amino acids (FIG. 8). These internal deletions span the entire coding sequence, except for the region between amino acids 140 to 234 which did not produce any stable derivatives. In general, each of the primers for the mutagenesis contained 20 - 25 nucleotides complementary to the regions immediately 5' and 3' of the desired deletion, as shown in Table 4, below.

TABLE 4

CONSTRUCT[1]	PRIMER
pCB.Egr-1Δ3-47	GTTCCGGCAG CACCGAGGAA TGCCATCCCG GACCAGCGAG (SEQ ID NO: 52)
pCB.Egr-1Δ50-93	AGGAC TCTGTGGTCA GGTGCTCATA GAGGAACTGG GGAGCCCCGT TGCTC (SEQ ID NO: 53)
pCB.Egr-1Δ94-139	AAAGTGTTGC CACTGTTGGG GGGTTGTTCG CTCGGCTCCC (SEQ ID NO: 54)
pCB.Egr-1Δ234-281	GATAGTGGAG TGAGCGAAGG GTACTGCAAG GCTGTCCTG (SEQ ID NO: 55)
pCB.Egr-1Δ284-330	GGCAA GCATATGGGC GTTCATGGGG CGAAGGCTGC TGGGTACGGTTCTCC (SEQ ID NO: 56)
pCB.Egr-1Δ331-374	AGGTGGTCAC TACGACTGAA GGGTGTCTTG CTGGGCCGGT (SEQ ID NO: 57)
pCB.Egr-1Δ377-419	TTGTCTGCTT TCTTGTCCTT ACTGAAGTTA CGCATGCAGA (SEQ ID NO: 58)
pCB.Egr-1Δ420-464	GTGGAGGAGC CAGGAGAGGA CTGTCTTAAA TGGATTTTGG (SEQ ID NO: 59)
pCB.Egr-1Δ465-509	GTTGAGGTGC TGAAGGAGCT GTAGGAAGTG GGCACAGGGG (SEQ ID NO: 60)
pCB.Egr-1Δ510-533	TTTTATTCCC TTTAGCAAAT GCTGACGCCC GCAGACGGGA (SEQ ID NO: 61)

Clones were typically screened by EcoRI/PvuI restriction; the deletion endpoints and reading frame were confirmed by dideoxy sequencing [Sanger, et al. 1977] with reagents from US Biochemical.

The construction of C-terminal deletions utilized site-directed mutagenesis to insert a stop codon followed by an XhoI site after amino acids 112, 138, 214, 240, 314, and 430. N-terminal deletions were

[1]The nomenclature of these internal deletion derivatives designates the first and last amino acids excised.

produced simply by excision of the internal XhoI fragment and religation in frame. The nomenclature is that deletions in from the N-terminus are designated by AN followed by the last amino acid deleted; C-terminal deletions are designated by AC and the last amino acid remaining. The reporters pA56foscat, containing the minimal murine c-fos promoter, and EBS1^{3}foscat with three copies of a high affinity Egr-1 binding site, have been described previously (16, 42).

In order to insert a 20 amino acid antigenic tag at the N-terminus of Egr-1, oligo-directed mutagenesis was used to create a unique NheI site at nucleotides 267-269 of pCB.Egr-1 with the primer 5'GAGACATCAA TTGCATCTCG GCCTTGCTAG CTGCCATCCC GGACCAGCGA GCTGGA 3' *(SEQ ID NO: 62)*. Annealed synthetic oligonucleotides coding for a portion of CMV glycoprotein A, followed by an XhoI site were inserted at the newly generated NheI site (amino acids 3 - 4 of Egr-1). The synthetic DNA encodes the 20 amino acids of the epitope Lys Gly Gln Lys Pro Asn Leu Leu Asp Arg Leu Arg His Arg Lys Asn Gly Tyr Arg His *(SEQ ID NO: 63)*, according to optimal human codon usage. The resulting plasmid was analyzed by sequencing and tested for protein expression, nuclear localization, and its ability to transactivate as compared to wild type Egr-1 in co-transfection assays. This plasmid, designated pCB.Egr-1.tag, was found to be equivalent to wild type Egr-1 in all assays, and was the parent for the subsequent construction of N- and C-terminal deletions.

EXAMPLE 10: Assay of deletion derivatives <u>for transcription activation</u>.

A series of deletion derivatives, each retaining the zinc finger domain, was assayed for transcriptional activity by transient transfection in HeLa and NIH3T3 cells (see FIG. 9). NIH3T3 and HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% calf serum. Cells were seeded at 8 x 10^5 cells per 100mm plate the day before transfection. The media was replaced 2 to 6 hours prior to transfection by calcium phosphate-mediated precipitation. Each precipitate included lμg of the internal reference pON260, a CMV driven β-galactosidase plasmid (67), or

3μg of PCH110, a SV40 driven β-galactosidase plasmid (Pharmacia), and remained on the cells for 16 - 20 hours. Forty-eight hours after transfection, cell extracts were prepared by freeze thaw lysis in 0.25M Tris-HC1 7.8 with protease inhibitors (1mM PMSF, 1μg/ml Pepstatin A, and 1μg/ml/ml Leupeptin).

Samples of transiently transfected cells prepared by freeze-thaw lysis were normalized both for β-galactosidase activity (as an internal control for transfection efficiency) and for total protein using the Biorad microprotein assay. Protein samples were separated by 10% SDS-PAGE (71) and transferred to PVDF membrane (Millipore). For the rabbit polyclonal anti-Egr-1 antiserum, a 1:4000 dilution of R5232-2 (6) and a 1:5000 dilution of horse radish peroxidase donkey anti-rabbit Ig (Amersham NA 934) were used. The monoclonal antibody CH28-2 reacts against a short region of human cytomegalovirus glycoprotein A. CH28-2 was used at a 1:20,000 dilution and the horse radish peroxidase sheep anti-mouse Ig (Amersham NA 931) at a 1:3000 dilution. Analysis using Amersham's enhanced chemiluminescence Western procedure (Amersham RPN 2106) required 1 to 10 minute exposures.

Chloramphenicol acetyltransferase (CAT) assays were performed according to Gorman *et al.* [Gorman, et al. 1982] with equal amounts of β-galactosidase activity in order to normalize for any variation in transfection efficiency. β-galactosidase activity was assayed with the substrate o-nitrophenyl-β-D-galactopyranoside (ONPG) as described [Rosenthal 1987].

Results of activation assays are shown in FIG. 9. A CAT reporter was constructed to include three copies of a high affinity Egr-1 binding site, 5' CGCCCCCGC 3' in front of the minimal murine c-fos promoter. The expression vector pCB.Egr-1, containing the full-length murine Egr-1 cDNA under control of the CMV early promoter/enhancer, activates transcription of this synthetic reporter ten-fold in NIH3T3 cells. This is not a cell type specific effect because similar data was obtained in Hela cells. Transactivation is absolutely dependent on the presence of Egr-l binding sites. As a negative control, an Egr-1 derivative deleted for part of the first and second

zinc fingers has been included; this mutant pCB.Egr-lA331-374 does not affect transcription.

Western analysis with monoclonal CH28-2 against the exogenous antigenic tag included in the deletion derivatives indicates that several of the larger N-terminal deletions are not well expressed. However, loss of sequence from the N-terminus to amino acid 214 resulted in diminished transcriptional activity to 5.5% (HeLa) or 14% (3T3) of wild type, with only a modest reduction in protein levels. A deletion removing the C-terminal 100 amino acids (AC430) reduced transcriptional activity in HeLa cells to about 20% of wild type but had no effect in NIH3T3 cells.

EXAMPLE 11: Fusion of functional domain sequences <u>to a yeast transcription factor</u>.

Functional domain sequences identified through deletion analysis were fused to the DNA-binding domain of the yeast transcription factor GAL4. Plasmid pSG424 [Sadowski, et al. 1988] encoding the DNA-binding domain of GAL4 driven by the SV40 early promoter/enhancer and followed by a polylinker and stop codons in all three reading frames provided the starting point for all GAL4-Egr-1 chimeras. (See FIG. 10.) Several domains of Egr-1 (aa 3-281, aa 3-138, aa 138-281, aa 420-533, aa 240-330, aa 281-330, and aa 281-314) were amplified by the polymerase chain reaction, digested with BamHI and XbaI, and cloned into the corresponding sites of pSG424 (see FIG. 10). The specified Egr-1 coding sequence is fused in-frame, C-terminal, to GAL4 amino acids 1 to 147, with seven synthetic amino acids at the junction. The amplified region and the junction of the construct were verified by dideoxy sequencing [Sanger, et al 1977].

Egr-1 binding was assayed as described in Cao *et al.* with the synthetic oligonucleotide EBS-1 5'-CGCCCT CGCCCCCGC GCCGGG-3' ***(SEQ ID NO: 64)***, labelled with Klenow and [α-^{32}P]CTP. The 140 bp HindIII/XbaI fragment from GAL4$_5$tkCAT, containing five copies of the 17 bp GAL4 binding site, was isolated on 3% NuSieve/1% Seaplaque agarose and purified with Mermaid (Bio 101). The fragment was labelled with [α-^{32}P]CTP and Klenow, and unincorporated nucleotides were removed with

Stratagene's Nuctrap column. Complexes were formed by incubating probe with 10μ1 of normalized extract in 20mM Tris-HC1/80mM NaCl/1mM dithiothreitol with 1μg poly dI-dC (Pharmacia 27-7880) and 10μg bovine serum albumin (Calbiochem 12659) in a total of 20μl for 30 minutes at room temperature. For cold competition experiments, a fifty-fold molar excess of unlabelled GAL4$_5$tkCAT HindIII/XbaI fragment was added.

The resulting chimeras were tested for their ability to transactivate a reporter containing five GAL4 binding sites in front of the E1b minimal promoter. Egr-1 amino acids 1 - 281 function to activate transcription about 100-fold in this assay; when subdivided into amino acids 3 - 138 138 - 281, these segments function as well as the intact domain. In addition, transcription is activated some 5-fold by the C-terminal region, amino acids 420 - 533. In all cases, activation is dependent on the presence of the GAL4 binding sites. Gel shift assays with the transiently transfected 3T3 cell extracts assure that each of the fusion proteins is expressed at comparable levels and is competent for DNA-binding. For example, the weak activity of GAL4-Egr-1(420-533) is not a result of low protein levels since this chimera is expressed as well as GAL4-Egr-1(3-281); GAL4-Egr-1(138-281) is over-expressed compared to GAL4-Egr-1(3-281), accounting for its superlative transactivation function.

The polypeptide corresponding to residues 3 - 281 (***SEQ ID NO: 3***) of the Egr-1 N-terminal domain is 30% serine-/threo-nine-/tyrosine-rich over a span of approximately 180 residues (amino acids 60 to 240), and includes several tracts of 5 - 7 consecutive serine or threonine residues. The large size of this activation domain may contribute to its potency relative to the smaller, previously described serine-/threonine-rich activator Pit-1 [Theill, et al. 1989]. Moreover, this transactivation domain is impervious to mutati in that substantial deletions in the extensive N-terminal domain do not impair transcriptional activity. A second, weaker activation domain (***SEQ ID NO: 10; see*** FIG. 7) is found in the C-terminus region of Egr-1, which has octapeptide repeats reminiscent of the phosphorylated Tyr Ser Pro Thr Ser

Pro Ser *(SEQ ID NO 40)* reiterations in the carboxy-terminal domain of the RNA polymerase II large subunit [Corden 1990].

EXAMPLE 12: Identification of a specific negative activation domain.

Deletion analyses were performed as described in Example 10, above. A small internal deletion immediately 5' of the zinc finger domain (Δ284 - - 330) was found to enhance transcription some five-fold in HeLa cells, indicating that a strong activation domain is encoded by the N-terminus of Egr-1, while a weaker transcriptional activity may reside in the C-terminus. The enhanced activation seen with deletion Δ284-330 is consistent with the loss of a region important for repression or negative regulation.

A fusion of GAL4(1-147) and the region of Egr-1 which, when deleted, enhanced activation was constructed according to methods described in Example 11. A GAL4 reporter with a high basal activity suitable for examining transcriptional repression was chosen ($GAL4_5$tkCAT). In this assay the GAL4-Egr-1(240-330) chimera repressed transcription ten-fold in a GAL4 binding site dependent manner. A fusion containing only 34 amino acids of Egr-1, GAL4-Egr-1(281-314), repressed transcription similarly. Gel shift analyses indicate that this repression is not due to overexpression of the GAL4-Egr-1 fusion proteins. Titration of the chimeric repressor (see FIG. 11) shows that as little as lμg GAL4-Egr-1(281-314) represses transcription more than five fold; even 10μg of the GAL4 DNA-binding domain does not recapitulate this effect. Therefore, in contrast to an extensive redundant activation domain, Egr-1 contains a repression function that can be precisely localized.

EXAMPLE 13: Mapping of the Egr-1 DNA binding domain.

The DNA binding activity of Egr-1, through homology to other zinc finger proteins such as TFIIIA [Shastry 1991] and Sp1 [Kadonaga, et al. 1988], should reside in the zinc finger domain. Gel mobility shift assays (see Example 11) with extracts from HeLa cells transiently transfected with the series of internal deletion derivatives show that only amino acids 331 - 419 of Egr-1 *(SEQ ID NO: 8)*, encoding the three zinc fingers, are required for specific DNA-binding. The deletion immediately N-terminal of the zinc finger domain (eight amino acids 5' of the first cysteine) has no effect on DNA-binding. The deletion C-terminal of the zinc fingers (four amino acids 3' of the last histidine) may slightly impair DNA-binding. Western analysis (see Example 10) with polyclonal anti-Egr-1 antisera indicates that the loss of DNA-binding activity with deletions within the zinc finger domain is not due to a reduction in protein expression.

EXAMPLE 14: Mapping of a bipartite nuclear localization signal.

Earlier work has shown that the Egr-1 gene product is localized to the nucleus [Cao et al. 1990; Day, et al. 1990; and Waters, et al. 1990]. To delineate the nuclear localization signal of Egr-1, the methods of subcellular fractionation and immunofluorescence of cells transfected with Egr-1 deletion derivatives, as well as in situ staining of Egr-l-β-galactosidase fusion proteins, were used.

CSH3T3 cells were plated on permanox chamber slides (Lab Tek 177429) and transfected as above. Cells were fixed in 4% formalin in 1X PBS (10 min, room temperature); permeabilized with acetone (7 min, -20 °C); and blocked in diluted normal goat serum (1 hour, room temperature). Cells were further incubated with a 1:500 dilution of anti-Egr-1 rabbit polyclonal R5232-T (1 hour, 37 °C) and a 1:200 dilution of fluorescein-conjugated goat anti-rabbit antiserum (l hour, room temperature, in the dark) from Caltag Labs (M30301). Coverslips were mounted with antifade mounting media, as described in Adams and Pringle [Adams, et al. 1984]. Photographs were taken at 40X magnification with Hypertech film.

Two transiently transfected 100mm plates of NIH3T3 cells were pooled for this analysis. Cells were lysed on ice in 100μl hypotonic buffer (25mM Tris-HCL 7.4/1mM MgC12/5mM KCl/1mM PMSF) with 0.5% NP-40 by pipetting briefly up and down. 2M sucrose was immediately added to 0.25M final, and nuclei were pelleted at 1000 x g in a microfuge for 1-2 minutes at 4 °C. Nuclei were washed in hypotonic buffer/0.5% NP-40 before lysis in 100μ1 hypotonic buffer/1% SDS. After addition of 2X protein sample buffer, nuclear fractions were sonicated.

Nuclear/cytoplasmic fractionation and Western analysis (see Example 10) of C-terminal deletions of Egr-1 revealed that while ΔC430 remained nuclear, further deletion in from the C-terminus produced derivatives that were predominantly cytoplasmic, for example ΔC314. These results were corroborated by indirect immunofluorescence microscopy. Deletion from the N-terminus to 314, or from the C-terminus to 430, produced derivatives that were nuclear. In contrast, C-terminal deletions past 430 were expressed throughout the cell. From these analyses, amino acids 315 to 419 (SEQ ID NOS. 6 and 7) appeared essential for proper nuclear localization. This region includes the three zinc fingers and adjacent sequences. Both the 5' flanking sequence Lys^{315} Pro Ser Arg Met Arg Lys Tyr Pro Asn Arg Pro Ser Lys Thr Pro^{330} ***(SEQ ID NO: 65)*** and the 3' flanking sequence Lys^{420} Asp Lys Lys Ala Asp Lys Ser Val Val^{429} ***(SEQ ID NO: 66)*** are characterized by a preponderance of basic residues. Immunofluorescence of internal deletion derivatives showed that each was properly targeted to the nucleus, suggesting that no single signal in Egr-1 directs nuclear accumulation.

In order to further delimit the residues within region 315-430 required for nuclear localization, segments of Egr-1 were fused to the large bacterial protein β-galactosidase (see FIG. 12). To create a β-galactosidase expression vector suitable for constructing N-terminal fusions, the 3.0 kb BamHI β-galactosidase fragment from pMC1871 was subcloned into the corresponding site of the CMV expression vector pCB6+. This fragment contains all of the coding sequence beginning with the tenth amino acid of β-galactosidase and is sufficient for its enzymatic activity. Secondly, a synthetic fragment containing an ATG in a Kozak context followed by the unique sites Bg1II, KpnI, HindIII, and XbaI was cloned into the Bg1II and XbaI sites to create pCB.β-GAL such that 1) the 5' most Bg1II site was destroyed and 2) the β-galactosidase coding sequence was in frame with the ATG. To create the fusion proteins, Egr-1 coding sequences have been amplified by PCR, cloned in frame into the unique Bg1II and XbaI sites of

pCB.β-GAL, and the resulting plasmids sequenced through the amplified regions.

To create pCB.Egr-1(315-330).β-GAL, complementary synthetic oligonucleotides were annealed with Bg1II and XbaI overhangs and cloned into the corresponding sites. Constructs containing the 5' basic region (aa 315 - 330) in conjunction with either the second finger, the third finger, the second and third fingers, or the H-C link of the third finger, were generated by cloning in frame into the XbaI site of pCB.Egr- 1(315-330).β-GAL. The construct containing the 5' basic flanking sequence with the H-C link of finger 3 and body of finger 1 was made such that there were no artifi[illegible] residues (at the restriction enzyme sites). Two annealed synthetic 90 encoding amino acids 315-330/389-395/338-363 were amplified with outside primers by PCR and cloned into the Bg1II and XbaI sites of pCB.β-gal. The construct with the 5' basic region, the H-C link of finger 1 and body of finger 3 was made by PCR amplification of two fragments such that no exogenous residues were introduced at the junction. The 5' fragment amplified residues 315 to 337, and amino acids 396 to 401 were included as a noncomplementary tail in the 3' primer. This DNA was annealed with the amplified fragment encoding residues 396 to 419 and the mixture reamplified with outside primers and cloned into the Bg1II and XbaI sites of pCB.β-gal.

The CMV promoter and a synthetic ATG drive expression of β-galactosidase coding sequence begin at codon ten. This protein retains its enzymatic activity, and staining of transiently transfected 3T3 cells with X-GAL show it to be distributed throughout the nucleus and cytoplasm as seen by others with similar constructs [31]. However, when the extensive finger domain with both 5' and 3' basic flanking sequences (aa 315 - 4[illegible] was fused N-terminal to β-galactosidase, the resulting chimera was fo[illegible] exclusively in the nucleus of transfected cells. This result indicates that the NLS of Egr-1 can function to confer nuclear localization on a heterologous bacterial protein. Because the C-terminus of Egr-1 had never been assayed for nuclear localization in the absence of amino acids 315 - 430 and might

contain an additional NLS, pCB.420-533.β-gal. was constructed. Subsequent analysis showed that the C-terminus of Egr-1 does not contain a redundant nuclear localization signal. Further analysis of residues 315 to 430 demonstrated that while the zinc finger domain alone, or in conjunction with the 3' basic sequence, was not sufficient for nuclear targeting, the finger domain with the adjacent 5' basic region localized β-galactosidase precisely to the nucleus. Yet the 5' basic sequence alone directs only partial nuclear accumulation with some residual staining in the cytoplasm, implying that the zinc fingers themselves participate in nuclear localization.

To define the region of the DNA-binding domain that cooperates with the 5' basic flanking sequence, chimeras with the 5' basic region (amino acids 315 - 330) and finger 1, fingers 2 and 3, finger 2, or finger 3 were constructed. While the 5' basic flanking sequence in combination with finger 3, or (to a lesser extent) finger 2, suffices for nuclear accumulation, finger 1 is unable to provide this function. The primary amino acid sequences of fingers 1 and 3, which bind to the same 3 nucleotide subsite, are quite similar (see FIG. 7). Principal differences lie in the H-C link region (a set of seven amino acids between the histidine of one finger and the cysteine of the following finger extremely well-conserved amongst Cys_2His_2 zinc finger proteins) and in several basic residues preceding the first histidine in finger 3.

These basic residues, which are also absent in finger 2, may account for the enhanced nuclear accumulation of constructs retaining finger 3 versus finger 2, but seem unlikely to explain the striking difference between the localization of fusions with finger 2 versus finger 1. Although the H-C links preceding fingers 2 and 3 conform well to the consensus, the H-C link of finger 1 does not. We asked whether the most important nuclear determinants lie in the H-C link or within the body of finger 3 by constructing chimeras between fingers 1 and 3 (in conjunction with the 5' basic sequence).

Staining with X-GAL shows a construct with the 5' basic region, the H-C link of finger 3, and the body of finger 1 to be cytoplasmic, while a

chimera containing the 5' basic region, the H-C link of finger 1, and the body of finger 3 accumulates somewhat in the nucleus. From this experiment we conclude that although the H-C link of finger 3 contributes marginally to nuclear localization, the most important nuclear determinants in finger 3 lie in the body of the finger.

While the above examples provide only limited illustration of *in vitro* and *in vivo* expression of DNA sequences of the invention, known recombinant techniques are readily applicable to development of a variety of procaryotic and eucaryotic expression systems for the large scale production of Egr proteins and even development of gene therapy regimens.

Knowledge of the specifically illustrated Egr-1 polypeptides of the invention has been demonstrated to provide a basis for preparation of highly useful antibodies, also provides a wealth of information concerning the nature of protein-nucleic acid interactions which, in turn, constitutes a basis for determination of significant early growth regulatory events. For example, and by analogy to steroid receptor protein structures, analysis of the structure of regions flanking the zinc fingers of Egr-1 and related proteins of the invention is expected to allow for identification of substances which may interact with the proteins to alter their DNA interactive capacities, and thus provide the basis for inhibition or augmentation of their regulatory functions. Moreover, information available concerning specific events of DNA interaction of Egr proteins of the invention will permit, e.g., identification and use of potential competitive inhibitors of these proteins.

It will be apparent from consideration of the foregoing illustrative examples that the present invention constitutes a substantial advance in the art and the achievement of a major goal in molecular biology, i.e., the characterization of genes which play a regulatory role in mammalian cell proliferation and differentiation. It will thus be understood that the information provided herein constitutes a basis for straightforward development of useful methods and materials not specifically the subject of the above examples. By way of illustration, possession of knowledge concerning the base sequence of cDNA and genomic DNA sequences encoding distinct

mouse Egr-1 and human EGR2 early growth regulatory proteins comprising histidine-cysteine zinc finger amino acid sequences makes possible the isolation of other such structurally related proteins. The substantial homology between the zinc finger regions of Egr-1 and EGR2 coupled with lack of homology in other protein regions, when considered in light of the ability of Egr-1 probes to localize to human chromosome 5 while EGR2 probes localize to human chromosome 10, essentially assures the straightforward isolation of a human gene (provisionally designated "human EGR1") which encodes a protein more closely homologous to Egr-1, and a mouse gene (Egr-2) encoding a protein more closely homologous to EGR2.

Because numerous modifications and variations in the practice of the present invention are expected to occur to those skilled in the art, only such limitations as appear in the appended claims should be placed thereon.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: METHODS AND MATERIALS RELATING TO THE FUNCTIONAL DOMAINSS OF DNA BINDING PROTEINS

(iii) NUMBER OF SEQUENCES: 67

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 533 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1

Met Ala Ala Ala Lys Ala Glu Met Gln Leu Met Ser Pro Leu Gln Ile
1 5 10 15

Ser Asp Pro Phe Gly Ser Phe Pro His Ser Pro Thr Met Asp Asn Tyr
20 25 30

Pro Lys Leu Glu Glu Met Met Leu Leu Ser Asn Gly Ala Pro Gln Phe
35 40 45

Leu Gly Ala Ala Gly Thr Pro Glu Gly Ser Gly Gly Asn Ser Ser Ser
50 55 60

Ser Thr Ser Ser Gly Gly Gly Gly Gly Gly Gly Ser Asn Ser Gly Ser
65 70 75 80

Ser Ala Phe Asn Pro Gln Gly Glu Pro Ser Glu Gln Pro Tyr Glu His
85 90 95

Leu Thr Thr Glu Ser Phe Ser Asp Ile Ala Leu Asn Asn Glu Lys Ala
100 105 110

Met Val Glu Thr Ser Tyr Pro Ser Gln Thr Thr Arg Leu Pro Pro Ile
115 120 125

Thr Tyr Thr Gly Arg Phe Ser Leu Glu Pro Ala Pro Asn Ser Gly Asn
130 135 140

Thr Leu Trp Pro Glu Pro Leu Phe Ser Leu Val Ser Gly Leu Val Ser
145 150 155 160

Met Thr Asn Pro Pro Thr Ser Ser Ser Ser Ala Pro Ser Pro Ala Ala
165 170 175

Ser Ser Ser Ser Ser Ala Ser Gln Ser Pro Pro Leu Ser Cys Ala Val
180 185 190

Pro Ser Asn Asp Ser Ser Pro Ile Tyr Ser Ala Ala Pro Thr Phe Pro
195 200 205

Thr Pro Asn Thr Asp Ile Phe Pro Glu Pro Gln Ser Gln Ala Phe Pro
210 215 220

Gly Ser Ala Gly Thr Ala Leu Gln Tyr Pro Pro Pro Ala Tyr Pro Ala
225 230 235 240

Thr Lys Gly Gly Phe Gln Val Pro Met Ile Pro Asp Tyr Leu Phe Pro
245 250 255

Gln Gln Gln Gly Asp Leu Ser Leu Gly Thr Pro Asp Gln Lys Pro Phe
260 265 270

Gln Gly Leu Glu Asn Arg Thr Gln Gln Pro Ser Leu Thr Pro Leu Ser
275 280 285

Thr Ile Lys Ala Phe Ala Thr Gln Ser Gly Ser Gln Asp Leu Lys Ala
290 295 300

Leu Asn Thr Thr Tyr Gln Ser Gln Leu Ile Lys Pro Ser Arg Met Arg
305 310 315 320

Lys Tyr Pro Asn Arg Pro Ser Lys Thr Pro Pro His Glu Arg Pro Tyr
325 330 335

Ala Cys Pro Val Glu Ser Cys Asp Arg Arg Phe Ser Arg Ser Asp Glu
340 345 350

Leu Thr Arg His Ile Arg Ile His Thr Gly Gln Lys Pro Phe Gln Cys
355 360 365

Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp His Leu Thr Thr His
370 375 380

Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys Gly
385 390 395 400

Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His Thr Lys Ile His
405 410 415

Leu Arg Gln Lys Asp Lys Lys Ala Asp Lys Ser Val Val Ala Ser Pro
420 425 430

Ala Ala Ser Ser Leu Ser Ser Tyr Pro Ser Pro Val Ala Thr Ser Tyr
435 440 445

Pro Ser Pro Ala Thr Thr Ser Phe Pro Ser Pro Val Pro Thr Ser Tyr
450 455 460

Ser Ser Pro Gly Ser Ser Thr Tyr Pro Ser Pro Ala His Ser Gly Phe
465 470 475 480

Pro Ser Pro Ser Val Ala Thr Thr Phe Ala Ser Val Pro Pro Ala Phe
485 490 495

Pro Thr Gln Val Ser Ser Phe Pro Ser Ala Gly Val Ser Ser Ser Phe
500 505 510

Ser Thr Ser Thr Gly Leu Ser Asp Met Thr Ala Thr Phe Ser Pro Arg
515 520 525

Thr Ile Glu Ile Cys
530

(2) INFORMATION FOR SEQ ID NO:2

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 456 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2

Met Met Thr Ala Lys Ala Val Asp Lys Ile Pro Val Thr Leu Ser Gly
1 5 10 15

Phe Val His Gln Leu Ser Asp Asn Ile Tyr Pro Val Glu Asp Leu Ala
20 25 30

Ala Thr Ser Val Thr Ile Phe Pro Asn Ala Glu Leu Gly Gly Pro Phe
35 40 45

Asp Gln Met Asn Gly Val Ala Gly Asp Gly Met Ile Asn Ile Asp Met
50 55 60

Thr Gly Glu Lys Arg Ser Leu Asp Leu Pro Tyr Pro Ser Ser Phe Ala
65 70 75 80

```
Pro Val Ser Ala Pro Arg Asn Gln Thr Phe Thr Tyr Met Gly Lys Phe
                85                  90                  95

Ser Ile Asp Pro Gln Tyr Pro Gly Ala Ser Cys Tyr Pro Glu Gly Ile
            100                 105                 110

Ile Asn Ile Val Ser Ala Gly Ile Leu Gln Gly Val Thr Ser Pro Ala
        115                 120                 125

Ser Thr Thr Ala Ser Ser Ser Val Thr Ser Ala Ser Pro Asn Pro Leu
    130                 135                 140

Ala Thr Gly Pro Leu Gly Val Cys Thr Met Ser Gln Thr Gln Pro Asp
145                 150                 155                 160

Leu Asp His Leu Tyr Ser Pro Pro Pro Pro Pro Pro Pro Tyr Ser Gly
                165                 170                 175

Cys Ala Gly Asp Leu Tyr Gln Asp Pro Ser Ala Phe Leu Ser Ala Ala
            180                 185                 190

Thr Thr Ser Thr Ser Ser Ser Leu Ala Tyr Pro Pro Pro Pro Ser Tyr
        195                 200                 205

Pro Ser Pro Lys Pro Ala Thr Asp Pro Gly Leu Phe Pro Met Ile Pro
    210                 215                 220

Asp Tyr Pro Gly Phe Phe Pro Ser Gln Cys Gln Arg Asp Leu His Gly
225                 230                 235                 240

Thr Ala Gly Pro Asp Arg Lys Pro Phe Pro Cys Pro Leu Asp Thr Leu
                245                 250                 255

Arg Val Pro Pro Pro Leu Thr Pro Leu Ser Thr Ile Arg Asn Phe Thr
            260                 265                 270

Leu Gly Gly Pro Ser Ala Gly Met Thr Gly Pro Gly Ala Ser Gly Gly
        275                 280                 285

Ser Glu Gly Pro Arg Leu Pro Gly Ser Ser Ser Ala Ala Ala Ala Ala
    290                 295                 300

Ala Ala Ala Ala Ala Tyr Asn Pro His His Leu Pro Leu Arg Pro Ile
305                 310                 315                 320

Leu Arg Pro Arg Lys Tyr Pro Asn Arg Pro Ser Lys Thr Pro Val His
                325                 330                 335

Glu Arg Pro Tyr Pro Cys Pro Ala Glu Gly Cys Asp Arg Arg Phe Ser
            340                 345                 350

Arg Ser Asp Glu Leu Thr Arg His Ile Arg Ile His Thr Gly His Lys
        355                 360                 365

Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp His
    370                 375                 380

Leu Thr Thr His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys
385                 390                 395                 400

Asp Tyr Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His
                405                 410                 415

Thr Lys Ile His Leu Arg Gln Lys Glu Arg Lys Ser Ser Ala Pro Ser
            420                 425                 430

Ala Ser Val Pro Ala Pro Ser Thr Ala Ser Cys Ser Gly Gly Val Gln
        435                 440                 445

Ala Trp Gly Tyr Pro Val Gln Gln
    450                 455
```


(2) INFORMATION FOR SEQ ID NO:3

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3

```
Met Ala Ala Ala Lys Ala Glu Met Gln Leu Met Ser Pro Leu Gln Ile
1               5                   10                  15

Ser Asp Pro Phe Gly Ser Phe Pro His Ser Pro Thr Met Asp Asn Tyr
            20                  25                  30

Pro Lys Leu Glu Glu Met Met Leu Leu Ser Asn Gly Ala Pro Gln Phe
        35                  40                  45

Leu Gly Ala Ala Gly Thr Pro Glu Gly Ser Gly Gly Asn Ser Ser Ser
    50                  55                  60

Ser Thr Ser Ser Gly Gly Gly Gly Gly Gly Gly Ser Asn Ser Gly Ser
65                  70                  75                  80

Ser Ala Phe Asn Pro Gln Gly Glu Pro Ser Glu Gln Pro Tyr Glu His
                85                  90                  95

Leu Thr Thr Glu Ser Phe Ser Asp Ile Ala Leu Asn Asn Glu Lys Ala
            100                 105                 110

Met Val Glu Thr Ser Tyr Pro Ser Gln Thr Thr Arg Leu Pro Pro Ile
        115                 120                 125

Thr Tyr Thr Gly Arg Phe Ser Leu Glu Pro Ala Pro Asn Ser Gly Asn
    130                 135                 140

Thr Leu Trp Pro Glu Pro Leu Phe Ser Leu Val Ser Gly Leu Val Ser
145                 150                 155                 160

Met Thr Asn Pro Pro Thr Ser Ser Ser Ser Ala Pro Ser Pro Ala Ala
                165                 170                 175

Ser Ser Ser Ser Ser Ala Ser Gln Ser Pro Pro Leu Ser Cys Ala Val
            180                 185                 190

Pro Ser Asn Asp Ser Ser Pro Ile Tyr Ser Ala Ala Pro Thr Phe Pro
        195                 200                 205

Thr Pro Asn Thr Asp Ile Phe Pro Glu Pro Gln Ser Gln Ala Phe Pro
    210                 215                 220

Gly Ser Ala Gly Thr Ala Leu Gln Tyr Pro Pro Pro Ala Tyr Pro Ala
225                 230                 235                 240

Thr Lys Gly Gly Phe Gln Val Pro Met Ile Pro Asp Tyr Leu Phe Pro
                245                 250                 255

Gln Gln Gln Gly Asp Leu Ser Leu Gly Thr Pro Asp Gln Lys Pro Phe
            260                 265                 270

Gln Gly Leu Glu Asn Arg Thr Gln Gln
        275                 280
```

(2) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 114 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4

```
Lys Asp Lys Lys Ala Asp Lys Ser Val Val Ala Ser Pro Ala Ala Ser
1               5                   10                  15

Ser Leu Ser Ser Tyr Pro Ser Pro Val Ala Thr Ser Tyr Pro Ser Pro
            20                  25                  30

Ala Thr Thr Ser Phe Pro Ser Pro Val Pro Thr Ser Tyr Ser Ser Pro
        35                  40                  45

Gly Ser Ser Thr Tyr Pro Ser Pro Ala His Ser Gly Phe Pro Ser Pro
    50                  55                  60

Ser Val Ala Thr Thr Phe Ala Ser Val Pro Pro Ala Phe Pro Thr Gln
65                  70                  75                  80

Val Ser Ser Phe Pro Ser Ala Gly Val Ser Ser Ser Phe Ser Thr Ser
                85                  90                  95

Thr Gly Leu Ser Asp Met Thr Ala Thr Phe Ser Pro Arg Thr Ile Glu
            100                 105                 110

Ile Cys
```

(2) INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5

```
Gln Pro Ser Leu Thr Pro Leu Ser Thr Ile Lys Ala Phe Ala Thr Gln
1               5                   10                  15

Ser Gly Ser Gln Asp Leu Lys Ala Leu Asn Thr Thr Tyr Gln Ser Gln
            20                  25                  30

Leu Ile
```

(2) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6

```
Lys Pro Ser Arg Met Arg Lys Tyr Pro Asn Arg Pro Ser Lys Thr Pro
1               5                   10                  15

Pro
```

(2) INFORMATION FOR SEQ ID NO:7

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7

```
Thr Gly Gln Lys Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser
1               5                   10                  15

Arg Ser Asp His Leu Thr Thr His Ile Arg Thr His Thr Gly Glu Lys
            20                  25                  30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu
        35                  40                  45

Arg Lys Arg His Thr Lys Ile His Leu Arg Gln
    50                  55
```

(2) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

```
Pro His Glu Arg Pro Tyr Ala Cys Pro Val Glu Ser Cys Asp Arg Arg
1               5                   10                  15

Phe Ser Arg Ser Asp Glu Leu Thr Arg His Ile Arg Ile His Thr Gly
            20                  25                  30

Gln Lys Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser
        35                  40                  45

Asp His Leu Thr Thr His Ile Arg Thr His Thr Gly Glu Lys Pro Phe
    50                  55                  60

Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys
65                  70                  75                  80

Arg His Thr Lys Ile His Leu Arg Gln
                85
```

(2) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 843 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

```
ATGGCAGCGG CCAAGGCCGA GATGCAATTG ATGTCTCCGC TGCAGATCTC TGACCCGTTC     60
GGCTCCTTTC CTCACTCACC CACCATGGAC AACTACCCCA AACTGGAGGA GATGATGCTG    120
CTGAGCAACG GGGCTCCCCA GTTCCTCGGT GCTGCCGGAA CCCCAGAGGG CAGCGGCGGT    180
AATAGCAGCA GCAGCACCAG CAGCGGGGGC GGTGGTGGGG GCGGCAGCAA CAGCGGCAGC    240
AGCGCCTTCA ATCCTCAAGG GGAGCCGAGC GAACAACCCT ATGAGCACCT GACCACAGAG    300
TCCTTTTCTG ACATCGCTCT GAATAATGAG AAGGCGATGG TGGAGACGAG TTATCCCAGC    360
CAAACGACTC GGTTGCCTCC CATCACCTAT ACTGGCCGCT TCTCCCTGGA GCCCGCACCC    420
AACAGTGGCA ACACTTTGTG GCCTGAACCC CTTTTCAGCC TAGTCAGTGG CCTCGTGAGC    480
ATGACCAATC CTCCGACCTC TTCATCCTCG GCGCCTTCTC CAGCTGCTTC ATCGTCTTCC    540
TCTGCCTCCC AGAGCCCGCC CCTGAGCTGT GCCGTGCCGT CCAACGACAG CAGTCCCATC    600
TACTCGGCTG CGCCCACCTT TCCTACTCCC AACACTGACA TTTTTCCTGA GCCCCAAAGC    660
CAGGCCTTTC CTGGCTCGGC AGGCACAGCC TTGCAGTACC CGCCTCCTGC CTACCCTGCC    720
ACCAAAGGTG GTTTCCAGGT TCCCATGATC CCTGACTATC TGTTTCCACA ACAACAGGGA    780
GACCTGAGCC TGGGCACCCC AGACCAGAAG CCCTTCCAGG GTCTGGAGAA CCGTACCCAG    840
CAG                                                                  843
```

(2) INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 342 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10

```
AAGGACAAGA AAGCAGACAA AAGTGTGGTG GCCTCCCCGG CTGCCTCTTC ACTCTCTTCT     60
TACCCATCCC CAGTGGCTAC CTCCTACCCA TCCCCTGCCA CCACCTCATT CCCATCCCCT    120
GTGCCCACTT CCTACTCCTC TCCTGGCTCC TCCACCTACC CATCTCCTGC GCACAGTGGC    180
TTCCCGTCGC CGTCAGTGGC CACCACCTTT GCCTCCGTTC CACCTGCTTT CCCCACCCAG    240
GTCAGCAGCT TCCCGTCTGC GGGCGTCAGC AGCTCCTTCA GCACCTCAAC TGGTCTTTCA    300
GACATGACAG CGACCTTTTC TCCCAGGACA ATTGAAATTT GC                       342
```

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11


```
CAGCCTTCGC TCACTCCACT ATCCACTATT AAAGCCTTCG CCACTCAGTC GGGCTCCCAG     60
GACTTAAAGG CTCTTAATAC CACCTACCAA TCCCAGCTCA TC                       102
```

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

```
AAACCCAGCC GCATGCGCAA GTACCCCAAC CGGCCCAGCA AGACACCC                  48
```

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

```
ACAGGCCAGA AGCCCTTCCA GTGTCGAATC TGCATGCGTA ACTTCAGTCG TAGTGACCAC     60
CTTACCACCC ACATCCGCAC CCACACAGGC GAGAAGCCTT TTGCCTGTGA CATTTGTGGG    120
AGGAAGTTTG CCAGGAGTGA TGAACGCAAG AGGCATACCA AAATCCATTT AAGACAG       177
```

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 267 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

```
CCCCATGAAC GCCCATATGC TTGCCCTGTC GAGTCCTGCG ATCGCCGCTT TTCTCGCTCG     60
GATGAGCTTA CCCGCCATAT CCGCATCCAC ACAGGCCAGA AGCCCTTCCA GTGTCGAATC    120
TGCATGCGTA ACTTCAGTCG TAGTGACCAC CTTACCACCC ACATCCGCAC CCACACAGGC    180
GAGAAGCCTT TTGCCTGTGA CATTTGTGGG AGGAAGTTTG CCAGGAGTGA TGAACGCAAG    240
AGGCATACCA AAATCCATTT AAGACAG                                        267
```

(2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3086 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

```
GGGGAGCCGC CGCCGCGATT CGCCGCCGCC GCCAGCTTCC GCCGCCGCAA GATCGGCCCC    60
TGCCCCAGCC TCCGCGGCAG CCCTGCGTCC ACCACGGGCC GCGGCTACCG CCAGCCTGGG   120
GGCCCACCTA CACTCCCCGC AGTGTGCCCC TGCACCCCGC ATGTAACCCG GCCAACCCCC   180
GGCGAGTGTG CCCTCAGTAG CTTCGGCCCC GGGCTGCGCC CACCACCCAA CATCAGTTCT   240
CCAGCTCGCT GGTCCGGGAT GGCAGCGGCC AAGGCCGAGA TGCAATTGAT GTCTCCGCTG   300
CAGATCTCTG ACCCGTTCGG CTCCTTTCCT CACTCACCCA CCATGGACAA CTACCCCAAA   360
CTGGAGGAGA TGATGCTGCT GAGCAACGGG GCTCCCCAGT TCCTCGGTGC TGCCGGAACC   420
CCAGAGGGCA GCGGCGGTAA TAGCAGCAGC AGCACCAGCA GCGGGGGCGG TGGTGGGGGC   480
GGCAGCAACA GCGGCAGCAG CGCCTTCAAT CCTCAAGGGG AGCCGAGCGA ACAACCCTAT   540
GAGCACCTGA CCACAGAGTC CTTTTCTGAC ATCGCTCTGA ATAATGAGAA GGCGATGGTG   600
GAGACGAGTT ATCCCAGCCA AACGACTCGG TTGCCTCCCA TCACCTATAC TGGCCGCTTC   660
TCCCTGGAGC CCGCACCCAA CAGTGGCAAC ACTTTGTGGC CTGAACCCCT TTTCAGCCTA   720
GTCAGTGGCC TCGTGAGCAT GACCAATCCT CCGACCTCTT CATCCTCGGC GCCTTCTCCA   780
GCTGCTTCAT CGTCTTCCTC TGCCTCCCAG AGCCCGCCCC TGAGCTGTGC CGTGCCGTCC   840
AACGACAGCA GTCCCATCTA CTCGGCTGCG CCCACCTTTC CTACTCCCAA CACTGACATT   900
TTTCCTGAGC CCCAAAGCCA GGCCTTTCCT GGCTCGGCAG GCACAGCCTT GCAGTACCCG   960
CCTCCTGCCT ACCCTGCCAC CAAAGGTGGT TTCCAGGTTC CCATGATCCC TGACTATCTG  1020
TTTCCACAAC AACAGGGAGA CCTGAGCCTG GGCACCCCAG ACCAGAAGCC CTTCCAGGGT  1080
CTGGAGAACC GTACCCAGCA GCCTTCGCTC ACTCCACTAT CCACTATTAA AGCCTTCGCC  1140
ACTCAGTCGG GCTCCCAGGA CTTAAAGGCT CTTAATACCA CCTACCAATC CCAGCTCATC  1200
AAACCCAGCC GCATGCGCAA GTACCCCAAC CGGCCCAGCA AGACACCCCC CCATGAACGC  1260
CCATATGCTT GCCCTGTCGA GTCCTGCGAT CGCCGCTTTT CTCGCTCGGA TGAGCTTACC  1320
CGCCATATCC GCATCCACAC AGGCCAGAAG CCCTTCCAGT GTCGAATCTG CATGCGTAAC  1380
TTCAGTCGTA GTGACCACCT TACCACCCAC ATCCGCACCC ACACAGGCGA GAAGCCTTTT  1440
GCCTGTGACA TTTGTGGGAG GAAGTTTGCC AGGAGTGATG AACGCAAGAG GCATACCAAA  1500
ATCCATTTAA GACAGAAGGA CAAGAAAGCA GACAAAAGTG TGGTGGCCTC CCCGGCTGCC  1560
TCTTCACTCT CTTCTTACCC ATCCCCAGTG GCTACCTCCT ACCCATCCCC TGCCACCACC  1620
TCATTCCCAT CCCCTGTGCC CACTTCCTAC TCCTCTCCTG GCTCCTCCAC CTACCCATCT  1680
CCTGCGCACA GTGGCTTCCC GTCGCCGTCA GTGGCCACCA CCTTTGCCTC CGTTCCACCT  1740
GCTTTCCCCA CCCAGGTCAG CAGCTTCCCG TCTGCGGGCG TCAGCAGCTC CTTCAGCACC  1800
TCAACTGGTC TTTCAGACAT GACAGCGACC TTTTCTCCCA GGACAATTGA AATTTGCTAA  1860
```



```
AGGGAATAAA AGAAAGCAAA GGGAGAGGCA GGAAAGACAT AAAAGCACAG GAGGGAAGAG  1920
ATGGCCGCAA GAGGGGCCAC CTCTTAGGTC AGATGGAAGA TCTCAGAGCC AAGTCCTTCT  1980
ACTCACGAGT AGAAGGACCG TTGGCCAACA GCCCTTTCAC TTACCATCCC TGCCTCCCCC  2040
GTCCTGTTCC CTTTGACTTC AGCTGCCTGA AACAGCCATG TCCAAGTTCT TCACCTCTAT  2100
CCAAAGGACT TGATTTGCAT GGTATTGGAT AAATCATTTC AGTATCCTCT CCATCACATG  2160
CCTGGCCCTT GCTCCCTTCA GCGCTAGACC ATCAAGTTGG CATAAAGAAA AAAAAATGGG  2220
TTTGGGCCCT CAGAACCCTG CCCTGCATCT TTGTACAGCA TCTGTGCCAT GGATTTTGTT  2280
TTCCTTGGGG TATTCTTGAT GTGAAGATAA TTTGCATACT CTATTGTATT ATTTGGAGTT  2340
AAATCCTCAC TTTGGGGGAG GGGGGAGCAA AGCCAAGCAA ACCAATGATG ATCCTCTATT  2400
TTGTGATGAC TCTGCTGTGA CATTAGGTTT GAAGCATTTT TTTTTTCAAG CAGCAGTCCT  2460
AGGTATTAAC TGGAGCATGT GTCAGAGTGT TGTTCCGTTA ATTTTGTAAA TACTGGCTCG  2520
ACTGTAACTC TCACATGTGA CAAAGTATGG TTTGTTTGGT TGGGTTTTGT TTTTGAGAAT  2580
TTTTTTGCCC GTCCCTTTGG TTTCAAAAGT TTCACGTCTT GGTGCCTTTT GTGTGACACG  2640
CCTTCCGATG GCTTGACATG CGCAGATGTG AGGGACACGC TCACCTTAGC CTTAAGGGGG  2700
TAGGAGTGAT GTGTTGGGGG AGGCTTGAGA GCAAAAACGA GGAAGAGGGC TGACCTGAGC  2760
TTTCGGTCTC CAGAATGTAA GAAGAAAAAA TTTAAACAAA AATCTGAACT CTCAAAAGTC  2820
TATTTTTCTA AACTGAAAAT GTAAATTTAT ACATCTATTC AGGAGTTGGA GTGTTGTGGT  2880
TACCTACTGA GTAGGCTGCA GTTTTTGTAT GTTATGAACA TGAAGTTCAT TATTTTGTGG  2940
TTTTATTTTA CTTTGTACTT GTGTTTGCTT AAACAAAGTA ACCTGTTTGG CTTATAAACA  3000
CATTGAATGC GCTCTATTGC CCATGGGATA TGTGGTGTGT ATCCTTCAGA AAAATTAAAA  3060
GGAAAAATAA AAAAAAAAAA AAAAAA                                       3086
```

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 3, 5, 7-10, 12-14, 16-20, 22-23, 25-27
(C) IDENTIFICATION METHOD: Xaa = any amino acid

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 4
(C) IDENTIFICATION METHOD: Xaa = Tyr or 3 Phe

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

```
Thr Gly Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Phe Xaa
1               5                   10                  15

Xaa Xaa Xaa Xaa Leu Xaa Xaa His Xaa Xaa Xaa His
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17

```
Pro His Glu Arg Pro Tyr Ala Cys Pro Val Glu Ser Cys Asp Arg Arg
1               5                   10                  15

Phe Ser Arg Ser Asp Glu Leu Thr Arg His Ile Arg Ile His
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18

```
Thr Gly Gln Lys Pro Phe Gln Cys
1               5
```

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19

```
Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp His Leu Thr Thr His
1               5                   10                  15

Ile Arg Thr His
            20
```

(2) INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20

```
Thr Gly Glu Lys Pro Phe Ala Cys
1               5
```


(2) INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21

```
Asp Ile Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His
1               5                   10                  15

Thr Lys Ile His
            20
```

(2) INFORMATION FOR SEQ ID NO:22

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22

```
Ser Arg Asp Lys Ser Phe Thr Cys Lys Ile
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:23

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23

```
Cys Ser Arg Ser Phe Gly Tyr Lys His Val Leu Gln Asn His Glu Arg
1               5                   10                  15

Thr His
```

(2) INFORMATION FOR SEQ ID NO:24

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24

```
Thr Gly Glu Lys Pro Phe Glu Cys Pro Glu
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:25

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25

```
Cys Asp Lys Arg Phe Thr Arg Asp His His Leu Lys Thr His Met Arg
1               5                   10                  15

Leu His
```

(2) INFORMATION FOR SEQ ID NO:26

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26

```
Thr Gly Glu Lys Pro Tyr His Cys Ser His
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:27

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27

```
Cys Asp Arg Gln Phe Val Gln Val Ala Asn Leu Arg Arg His Leu Arg
1               5                   10                  15

Val His
```


(2) INFORMATION FOR SEQ ID NO:28

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28

```
Thr Gly Glu Arg Pro Tyr Thr Cys Glu Ile
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:29

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29

```
Cys Asp Gly Lys Phe Ser Asp Ser Asn Gln Leu Lys Ser His Met Leu
1               5                   10                  15

Val His
```

(2) INFORMATION FOR SEQ ID NO:30

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30

```
Thr Gly Glu Lys Pro Phe Pro Cys Lys Glu Glu Gly Cys Glu Lys Gly
1               5                   10                  15

Phe Thr Ser Leu His His Leu Thr Arg His Ser Leu Thr His
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO:31

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2811 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31

```
TTTTTTTTTT TGGTGTGTGT GGTGGTTGTT TTTAAGTGTG GAGGGCAAAA GGAGATACCA     60
TCCCAGGCTC AGTCCAACCC CTCTCCAAAA CGTGTCTTTT CTGACACTCC AGGTAGCGAG    120
GGAGTTGGGT CTCCAGGTTG TGCGAGGAGC AAATGATGAC CGCCAAGGCC GTAGACAAAA    180
TCCCAGTAAC TCTCAGTGGT TTTGTGCACC AGCTGTCTGA CAACATCTAC CCGGTGGAGG    240
ACCTCGCCGC CACGTCGGTG ACCATCTTTC CCAATGCCGA ACTGGGAGGC CCCTTTGACC    300
AGATGAACGG AGTGGCCGGA GATGGCATGA TCAACATTGA CATGACTGGA GAGAAGAGGT    360
CGTTGGATCT CCCATATCCC AGCAGCTTTG CTCCCGTCTC TGCACCTAGA AACCAGACCT    420
TCACTTACAT GGGCAAGTTC TCCATTGACC CACAGTACCC TGGTGCCAGC TGCTACCCAG    480
AAGGCATAAT CAATATTGTG AGTGCAGGCA TCTTGCAAGG GGTCACTTCC CCAGCTTCAA    540
CCACAGCCTC ATCCAGCGTC ACCTCTGCCT CCCCCAACCC ACTGGCCACA GGACCCCTGG    600
GTGTGTGCAC CATGTCCCAG ACCCAGCCTG ACCTGGACCA CCTGTACTCT CCGCCACCGC    660
CTCCTCCTCC TTATTCTGGC TGTGCAGGAG ACCTCTACCA GGACCCTTCT GCGTTCCTGT    720
CAGCAGCCAC CACCTCCACC TCTTCCTCTC TGGCCTACCC ACCACCTCCT TCCTATCCAT    780
CCCCCAAGCC AGCCACGGAC CCAGGTCTCT TCCCAATGAT CCCAGACTAT CCTGGATTCT    840
TTCCATCTCA GTGCCAGAGA GACCTACATG GTACAGCTGG CCCAGACCGT AAGCCCTTTC    900
CCTGCCCACT GGACACCCTG CGGGTGCCCC CTCCACTCAC TCCACTCTCT ACAATCCGTA    960
ACTTTACCCT GGGGGGCCCC AGTGCTGGGA TGACCGGACC AGGGGCCAGT GGAGGCAGCG   1020
AGGGACCCCG GCTGCCTGGT AGCAGCTCAG CAGCAGCAGC AGCCGCCGCC GCCGCCGCCT   1080
ATAACCCACA CCACCTGCCA CTGCGGCCCA TTCTGAGGCC TCGCAAGTAC CCCAACAGAC   1140
CCAGCAAGAC GCCGGTGCAC GAGAGGCCCT ACCCGTGCCC AGCAGAAGGC TGCGACCGGC   1200
GGTTCTCCCG CTCTGACGAG CTGACACGGC ACATCCGAAT CCACACTGGG CATAAGCCCT   1260
TCCAGTGTCG GATCTGCATG CGCAACTTCA GCCGCAGTGA CCACCTCACC ACCCATATCC   1320
GCACCCACAC CGGTGAGAAG CCCTTCGCCT GTGACTACTG TGGCCGAAAG TTTGCCCGGA   1380
GTGATGAGAG GAAGCGCCAC ACCAAGATCC ACCTGAGACA GAAAGAGCGG AAAAGCAGTG   1440
CCCCCTCTGC ATCGGTGCCA GCCCCCTCTA CAGCCTCCTG CTCTGGGGGC GTGCAGGCCT   1500
GGGGGTACCC TGTGCAGCAG TAACAGCAGC AGTCTTGGCG GAGGGCCGCT CGCCCCTTGC   1560
TCCTCTCGGA CCCGGACACC TTGAGATGAG ACTCAGGCTG ATACACCAGC TCCCAAAGGT   1620
CCCGGAGGCC CTTTGTCCAC TGGAGCTGCA CAACAAACAC TACCACCCTT TCCTGTCCCT   1680
CTCTCCCTTT GTTGGGCAAA GGGCTTTGGT GGAGCTAGCA CTGCCCCCTT TCCACCTAGA   1740
AGCAGGTTCT TCCTAAAACT TAGCCCATTC TAGTCTCTCT TAGGTGAGTT GACTATCAAC   1800
CCAAGGCAAA GGGGAGGCTC AGAAGGAGGT GGTGTGGGGA TCCCCTGGCC AAGAGGGCTG   1860
```



```
AGGTCTGACC CTGCTTTAAA GGGTTGTTTG ACTAGGTTTT GCTACCCCAC TTCCCCTTAT   1920
TTTGACCCAT CACAGGTTTT TGACCCTGGA TGTCAGAGTT GATCTAAGAC GTTTTCTACA   1980
ATAGGTTGGG AGATGCTGAT CCCTTCAAGT GGGGACAGCA AAAAGACAAG CAAAACTGAT   2040
GTGCACTTTA TGGCTTGGGA CTGATTTGGG GGACATTGTA CAGTGAGTGA AGTATAGCCT   2100
TTATGCCACA CTCTGTGGCC CTAAAATGGT GAATCAGAGC ATATCTAGTT GTCTCAACCC   2160
TTGAAGCAAT ATGTATTATA TACTCAGAGA ACAGAAGTGC AATGTGATGG GAGGAACGTA   2220
GCAATATCTG CTCCTTTTCG AGTTGTTTGA GAAATGTAGG CTATTTTTTC AGTGTATATC   2280
CACTCAGATT TTGTGTATTT TTGATGTACC CACACTGTTC TCTAAATTCT GAATCTTTGG   2340
GAAAAAATGT AAAGCATTTA TGATCTCAGA GGTTAACTTA TTTAAGGGGG ATGTACATAT   2400
TCTCTGAAAC TAGGATGCAT GCAATTGTGT TGGAAGTGTC CTTGGTCGCC TTGTGTGATG   2460
TAGACAAATG TTACAAGGCT GCATGTAAAT GGGTTGCCTT ATTATGGAGA AAAAAATCAC   2520
TCCCTGAGTT TAGTATGGCT GTATATTTAT GCCTATTAAT ATTTCAAATT TTTTTTTAGA   2580
GTATATTTTT GTATGCTTTG TTTTGTGACT TAAAAGTGTT ACCTTTGTAG TCAAATTTCA   2640
GATAAGAATG TACATAATGT TACCGGAGCT GATTGTTTGG TCATTAGCTC TTAATAGTTG   2700
TGAAAAAATA AATCTATTCT AACGCAAAAC CACTAACTGA AGTTCAGATA TAATGGATGG   2760
TTTGTGACTA TAGTGTATAA ATACTTTTCA ACAAAAAAAA AAAAAAAAAA A            2811
```

(2) INFORMATION FOR SEQ ID NO:32

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1200 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32

```
ACGGAGGGAA TAGCCTTTCG ATTCTGGGTG GTGCATTGGA AGCCCCAGGC TCTAAAACCC     60
CCAACCTACT GACTGGTGGC CGAGTATGCA CCCGACTGCT AGCTAGGCAG TGTCCCAAGA    120
ACCAGTAGCC AAATGTCTTG GCCTCAGTTT TCCCGGTGAC ACCTGGAAAG TGACCCTGCC    180
ATTAGTAGAG GCTCAGGTCA GGGCCGCGCC TCTCCTGGGC GGCCTCTGCC CTAGCCCGCC    240
CTGCCGCTCC TCCTCTCCGC AGGCTCGCTC CCACGGTCCC CGAGGTGGGC GGGTGAGCCC    300
AGGATGACGG CTGTAGAACC CCGGCCTGAC TCGCCCTCGC CCCCGCGCCG GGCCTGGGCT    360
TCCCTAGCCC AGCTCGCACC CGGGGGCCGT CGGAGCCGCC GCGCGCCCAG CTCTACGCGC    420
CTGGCCCTCC CCACGCGGGC GTCCCCGACT CCCGCGCGCG CTCAGGCTCC CAGTTGGGAA    480
CCAAGGAGGG GGAGGATGGG GGGGGGGGTG TGCGCCGACC CGGAAACGCC ATATAAGGAG    540
CAGGAAGGAT CCCCCGCCGG AACAGACCTT ATTTGGGCAG CGCCTTATAT GGAGTGGCCC    600
AATATGGCCC TGCCGCTTCC GGCTCTGGGA GGAGGGGCGA GCGGGGGTTG GGGCGGGGGC    660
AAGCTGGGAA CTCCAGGCGC CTGGCCCGGG AGGCCACTGC TGCTGTTCCA ATACTAGGCT    720
TTCCAGGAGC CTGAGCGCTC GCGATGCCGG AGCGGGTCGC AGGGTGGAGG TGCCCACCAC    780
TCTTGGATGG GAGGGCTTCA CGTCACTCCG GGTCCTCCCG GCCGGTCCTT CCATATTAGG    840
```

```
GCTTCCTGCT TCCCATATAT GGCCATGTAC GTCACGGCGG AGGCGGGCCC GTGCTGTTCC    900
AGACCCTTGA AATAGAGGCC GATTCGGGGA GTCGCGAGAG ATCCCAGCGC GCAGAACTTG    960
GGGAGCCGCC GCCGCGATTC GCCGCCGCCG CCAGCTTCCG CCGCCGCAAG ATCGGCCCCT   1020
GCCCCAGCCT CCGCGGCAGC CCTGCGTCCA CCACGGGCCG CGGCTACCGC CAGCCTGGGG   1080
GCCCACCTAC ACTCCCCGCA GTGTGCCCCT GCACCCCGCA TGTAACCCGG CCAACCCCCG   1140
GCGAGTGTGC CCTCAGTAGC TTCGGCCCCG GGCTGCGCCC ACCACCCAAC ATCAGTTCTC   1200
```

(2) INFORMATION FOR SEQ ID NO:33

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33

```
Gln Pro Ser Leu Thr Pro Leu Ser Thr Ile Lys Ala Phe Ala Thr Gln
1               5                   10                  15

Ser Gly Ser Gln Asp Leu Lys Ala Leu Asn Thr Thr Tyr Gln Ser Gln
            20                  25                  30

Leu Ile Lys Pro Ser Arg Met Arg Lys Tyr Pro Asn Arg Pro Ser Lys
        35                  40                  45

Thr Pro Pro His Glu Arg Pro Tyr Ala Cys Pro Val Glu Ser Cys Asp
    50                  55                  60

Arg Arg Phe Ser Arg Ser Asp Glu Leu Thr Arg His Ile Arg Ile His
65                  70                  75                  80

Thr Gly Gln Lys Pro Phe Gln Cys
                85
```

(2) INFORMATION FOR SEQ ID NO:34

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34

```
Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp His Leu Thr Thr His
1               5                   10                  15

Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys
            20                  25
```


(2) INFORMATION FOR SEQ ID NO:35

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35

```
Asp Ile Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His
1               5                   10                  15

Thr Lys Ile His Leu Arg Gln Lys Asp Lys Lys Ala Asp Lys Ser Val
            20                  25                  30

Val Ala Ser
        35
```

(2) INFORMATION FOR SEQ ID NO:36

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36

```
Gln Arg Gln Leu Thr Val Ser Pro Glu Leu Pro Gly Ile Arg Arg Arg
1               5                   10                  15

Tyr Pro Gly Glu Phe Glu Leu
            20
```

(2) INFORMATION FOR SEQ ID NO:37

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 87 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37

```
CAAAGACAGT TGACTGTATC GCCGGAATTC CCGGGGATCC GTCGACGGTA CCCCGGGGAA      60
TTCGAGCTCT AGATAAGTAA TGATTCA                                         87
```

(2) INFORMATION FOR SEQ ID NO:38

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38

```
His Leu Arg Gln Lys Asp Lys Lys Ala Asp Lys Ser Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:39

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39

```
Cys Gly Arg Lys Phe Ala Arg Ser Asp Glu Arg Lys Arg His Thr Lys
1               5                   10                  15

Ile
```

(2) INFORMATION FOR SEQ ID NO:40

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40

```
Tyr Ser Pro Thr Ser Pro Ser
1               5
```

(2) INFORMATION FOR SEQ ID NO:41

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 4
(C) IDENTIFICATION METHOD: Xaa = Arg or Lys

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 6
(C) IDENTIFICATION METHOD: Xaa = Phe or Tyr

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 7
(C) IDENTIFICATION METHOD: Xaa = Any amino acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41

```
Thr Gly Glu Xaa Pro Xaa Xaa
1               5
```

(2) INFORMATION FOR SEQ ID NO:42

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43

```
Asn Ser Ser Ser Ser Thr Ser Ser
1               5
```

(2) INFORMATION FOR SEQ ID NO:43

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43

```
Asn Asn Ser Ser Ser Ser Ser Ser Ser Ser Ser
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:44

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44

```
GATGTCCATA TTAGGACATC                                    20
```

(2) INFORMATION FOR SEQ ID NO:45

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45

GATCCCCTAA TTATGGGGAT C 21

(2) INFORMATION FOR SEQ ID NO:46

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46

GATGGCCATA TTAGGCCATC 20

(2) INFORMATION FOR SEQ ID NO:47

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47

TCCTTCCATA TTAGGCCTTC 20

(2) INFORMATION FOR SEQ ID NO:48

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48

AATATGGCCC TG 12

(2) INFORMATION FOR SEQ ID NO:49

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49

CAGCGCCTTA TATGGAGTGG 20

(2) INFORMATION FOR SEQ ID NO:50

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50

ACAGACCTTA TTTGGGCAGC 20

(2) INFORMATION FOR SEQ ID NO:51

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51

AAACGCCATA TAAGGAGCAG 20

(2) INFORMATION FOR SEQ ID NO:52

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52

GTTCCGGCAG CACCGAGGAA TGCCATCCCG GACCAGCGAG 40

(2) INFORMATION FOR SEQ ID NO:53

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53

AGGACTCTGT GGTCAGGTGC TCATAGAGGA ACTGGGGAGC CCCGTTGCTC 50

(2) INFORMATION FOR SEQ ID NO:54

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54

AAAGTGTTGC CACTGTTGGG GGGTTGTTCG CTCGGCTCCC 40

(2) INFORMATION FOR SEQ ID NO:55

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56

GATAGTGGAG TGAGCGAAGG GTACTGCAAG GCTGTGCCTG 40

(2) INFORMATION FOR SEQ ID NO:56

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56

GGCAAGCATA TGGGCGTTCA TGGGGCGAAG GCTGCTGGGT ACGGTTCTCC 50

(2) INFORMATION FOR SEQ ID NO:57

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57

AGGTGGTCAC TACGACTGAA GGGTGTCTTG CTGGGCCCGG T 41

(2) INFORMATION FOR SEQ ID NO:58

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58

TTGTCTGCTT TCTTGTCCTT ACTGAAGTTA CGCATGCAGA 40

(2) INFORMATION FOR SEQ ID NO:14:59

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59

GTGGAGGAGC CAGGAGAGGA CTGTCTTAAA TGGATTTTGG 40

(2) INFORMATION FOR SEQ ID NO:60

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60

GTTGAGGTGC TGAAGGAGCT GTAGGAAGTG GGCACAGGGG 40

(2) INFORMATION FOR SEQ ID NO:61

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61

```
TTTTATTCCC TTTAGCAATG CTGACGCCCG CAGACGGGA                    39
```

(2) INFORMATION FOR SEQ ID NO:62

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 56 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62

```
GAGACATCAA TTGCATCTCG GCCTTGCTAG CTGCCATCCC GGACCAGCGA GCTGGA        56
```

(2) INFORMATION FOR SEQ ID NO:63

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63

```
Lys Gly Gln Lys Pro Asn Leu Leu Asp Arg Leu Arg His Arg Lys Asn
1               5                   10                  15

Gly Tyr Arg His
            20
```

(2) INFORMATION FOR SEQ ID NO:64

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64

```
CGCCCTCGCC CCCGCGCCGG G                                       21
```

(2) INFORMATION FOR SEQ ID NO:65

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65

```
Lys Pro Ser Arg Met Arg Lys Tyr Pro Asn Arg Pro Ser Lys Thr Pro
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:66

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66

```
Lys Asp Lys Lys Ala Asp Lys Ser Val Val
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:67

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Xaa
(B) LOCATION: 2-5, 7-9, 11-15, 17-18, 20-22
(C) IDENTIFICATION METHOD: Xaa = Any amino acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67

```
Cys Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Leu
1               5                   10                  15

Xaa Xaa His Xaa Xaa Xaa His
            20
```

WHAT IS CLAIMED IS:

1. An isolated and purified mammalian early growth regulatory polypeptide comprising a functional domain, wherein said functional domain performs a function of activation of transcription, repression of transcription, nuclear localization, or polynucleotide binding.

2. The isolated and purified polypeptide of Claim 1 comprising one functional domain.

3. The isolated and purified polypeptide of Claim 1 comprising the amino acid residue sequence of SEQ ID NO: 1 or SEQ ID NO:2.

4. The isolated and purified polypeptide of Claim 2 comprising the amino acid residue sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 8.

5. A polypeptide product of the in vitro or in vivo expression of a polypeptide-encoding region of a purified and isolated polynucleotide sequence, wherein said polypeptide product is a fusion polypeptide of a mammalian early growth regulatory polypeptide that comprises one or more functional domains, and part or all of a heterologous protein.

6. An isolated and purified polynucleotide that encodes a mammalian early growth regulatory polypeptide other than an intact mammalian early growth regulatory polypeptide, the polypeptide comprising a functional domain performing the function of activation of transcription, repression of transcription, nuclear localization, or polynucleotide binding.

7. The isolated and purified polynucleotide of Claim 6 wherein the polypeptide comprises the amino acid residue sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, or SEQ ID NO: 8.

8. The isolated and purified polynucleotide of Claim 6 wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 14.

9. An isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 14, wherein said polynucleotide hybridizes to a polynucleotide that encodes a mammalian early growth regulatory polypeptide.

10. An isolated and purified polynucleotide that comprises a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 9, said isolated and purified polynucleotide hybridizing to SEQ ID NO: 9 or a complement of SEQ ID NO: 9.

11. An isolated and purified polynucleotide that comprises a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 11, said isolated and purified polynucleotide hybridizing to SEQ ID NO: 11 or a complement of SEQ ID NO: 11.

12. An isolated and purified polynucleotide that encodes a mammalian early growth regulatory polypeptide, said polynucleotide preparable by a process comprising the steps of:

a. constructing a library of cDNA clones from a cell that expresses said polypeptide;

b. screening the library with a labelled cDNA probe prepared from RNA that encodes said polypeptide; and

c. selecting a clone that hybridizes to the probe.

13. The isolated and purified polynucleotide of Claim 12 wherein preparable by is prepared by.

14. An expression vector comprising the polynucleotide of Claim 6.

15. The expression vector of Claim 14 further comprising an enhancer/promoter operatively linked to the polynucleotide.

16. The expression vector of Claim 15 wherein the enhancer/promoter is a cytomegalovirus enhancer/promoter.

17. A cell transfected with a polynucleotide that encodes a mammalian early growth regulatory polypeptide.

18. A process of preparing the polypeptide of Claim 1 comprising:

a. transfecting a cell with a polynucleotide that encodes the polypeptide to produce a transformed cell; and

b. maintaining the transformed cell under biological conditions sufficient for expression of the polypeptide.

19. A polypeptide prepared by the process of Claim 18.

20. A process for regulation of transcription of a polynucleotide that encodes a mammalian early growth regulatory polypeptide, said process comprising the steps of:

a. transfecting a cell that contains the polynucleotide with the expression vector of Claim 14; and

b. maintaining the cell under physiological conditions sufficient to regulate transcription.

21. A pharmaceutical composition comprising the polypeptide of Claim 1 and a physiologically acceptable carrier.

22. A pharmaceutical composition comprising a polynucleotide that encodes the polypeptide of Claim 1 and a physiologically acceptable carrier.

23. An antibody immunoreactive with a mammalian early growth regulatory polypeptide.

24. The antibody according to claim 23 that is a monoclonal antibody.

25. A diagnostic assay kit for detecting the presence of a mammalian early growth regulatory polypeptide, said kit comprising a first container containing, in an amount sufficient to perform at least one assay, a first antibody capable of immunoreacting with said polypeptide.

26. The assay kit of Claim 25, wherein said first antibody is affixed to a solid support.

27. The assay kit of Claim 25 further comprising a second container containing a second antibody that immunoreacts with said first antibody.

28. The assay kit of Claim 27, wherein said first and second antibodies are monoclonal antibodies.

29. The assay kit of Claim 27, wherein said first and second antibodies comprise an indicator.

30. The assay kit of Claim 29, wherein said indicator is a radioactive label or an enzyme.

31. A diagnostic assay kit for detecting the presence, in a biological sample, of a first polynucleotide that encodes a mammalian early growth regulatory polypeptide, said kit comprising a first container that contains a

second polynucleotide identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 14.

32. A diagnostic assay kit for detecting the presence, in a biological sample, of an antibody immunoreactive with a mammalian early growth regulatory polypeptide, said kit comprising a first container containing, in an amount sufficient to perform at least one assay, a mammalian early growth regulatory polypeptide that immunoreacts with said antibody.

33. A process of detecting a mammalian early growth regulatory polypeptide, the process comprising immunoreacting the polypeptide with the antibody of claim 23 to form an antibody-polypeptide conjugate, and detecting the conjugate.

34. A process of detecting a messenger RNA transcript that encodes a mammalian early growth regulatory polypeptide, the process comprising the steps of:

a. hybridizing the messenger RNA transcript with a polynucleotide sequence that encodes a mammalian early growth regulatory polypeptide to form a duplex; and

b. detecting the duplex.

35. A process of detecting a DNA molecule that encodes for a mammalian early growth regulatory polypeptide, the process comprising the steps of:

a. hybridizing the DNA molecule with a polynucleotide that encodes a mammalian early growth regulatory polypeptide to form a duplex; and

b. detecting the duplex.

```
10         20         30         40         50         60
GGGGAGCCGCCGCCGCGATTCGCCGCCGCCGCCAGCTTCCGCCGCCGCAAGATCGGCCCC

70         80         90         100        110        120
TGCCCCAGCCTCCGCGGCAGCCCTGCGTCCACCACGGGCCGCGGCTACCGCCAGCCTGGG

130        140        150        160        170        180
GGCCCACCTACACTCCCCGCAGTGTGCCCCTGCACCCCGCATGTAACCCGGCCAACCCCC

190        200        210        220        230        240
GGCGAGTGTGCCCTCAGTAGCTTCGGCCCCGGGCTGCGCCCACCACCCAACATCAGTTCT

250        260        270        280        290        300
CCAGCTCGCTGGTCCGGGATGGCAGCGGCCAAGGCCGAGATGCAATTGATGTCTCCGCTG
                   MetAlaAlaAlaLysAlaGluMetGlnLeuMetSerProLeu

310        320        330        340        350        360
CAGATCTCTGACCCGTTCGGCTCCTTTCCTCACTCACCCACCATGGACAACTACCCCAAA
GlnIleSerAspProPheGlySerPheProHisSerProThrMetAspAsnTyrProLys

370        380        390        400        410        420
CTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGTGCTGCCGGAACC
LeuGluGluMetMetLeuLeuSerAsnGlyAlaProGlnPheLeuGlyAlaAlaGlyThr
```

FIG. 1.1


```
430        440        450        460        470        480
CCAGAGGGCAGCGGCGGTAATAGCAGCAGCAGCACCAGCAGCGGGGGCGGTGGTGGGGGC
ProGluGlySerGlyGlyAsnSerSerSerSerThrSerSerGlyGlyGlyGlyGlyGly

490        500        510        520        530        540
GGCAGCAACAGCGGCAGCAGCGCCTTCAATCCTCAAGGGGAGCCGAGCGAACAACCCTAT
GlySerAsnSerGlySerSerAlaPheAsnProGlnGlyGluProSerGluGlnProTyr

550        560        570        580        590        600
GAGCACCTGACCACAGAGTCCTTTTCTGACATCGCTCTGAATAATGAGAAGGCGATGGTG
GluHisLeuThrThrGluSerPheSerAspIleAlaLeuAsnAsnGluLysAlaMetVal

610        620        630        640        650        660
GAGACGAGTTATCCCAGCCAAACGACTCGGTTGCCTCCCATCACCTATACTGGCCGCTTC
GluThrSerTyrProSerGlnThrThrArgLeuProProIleThrTyrThrGlyArgPhe

670        680        690        700        710        720
TCCCTGGAGCCCGCACCCAACAGTGGCAACACTTTGTGGCCTGAACCCCTTTTCAGCCTA
SerLeuGluProAlaProAsnSerGlyAsnThrLeuTrpProGluProLeuPheSerLeu

730        740        750        760        770        780
GTCAGTGGCCTCGTGAGCATGACCAATCCTCCGACCTCTTCATCCTCGGCGCCTTCTCCA
ValSerGlyLeuValSerMetThrAsnProProThrSerSerSerSerAlaProSerPro

790        800        810        820        830        840
GCTGCTTCATCGTCTTCCTCTGCCTCCCAGAGCCCGCCCCTGAGCTGTGCCGTGCCGTCC
AlaAlaSerSerSerSerSerAlaSerGlnSerProProLeuSerCysAlaValProSer
```

FIG. 1.2

850 860 870 880 890 900
AACGACAGCAGTCCCATCTACTCGGCTGCGCCCACCTTTCCTACTCCCAACACTGACATT
AsnAspSerSerProIleTyrSerAlaAlaProThrPheProThrProAsnThrAspIle

910 920 930 940 950 960
TTTCCTGAGCCCCAAAGCCAGGCCTTTCCTGGCTCGGCAGGCACAGCCTTGCAGTACCCG
PheProGluProGlnSerGlnAlaPheProGlySerAlaGlyThrAlaLeuGlnTyrPro

970 980 990 1000 1010 1020
CCTCCTGCCTACCCTGCCACCAAAGGTGGTTTCCAGGTTCCCATGATCCCTGACTATCTG
ProProAlaTyrProAlaThrLysGlyGlyPheGlnValProMetIleProAspTyrLeu

1030 1040 1050 1060 1070 1080
TTTCCACAACAACAGGGAGACCTGAGCCTGGGCACCCCAGACCAGAAGCCCTTCCAGGGT
PheProGlnGlnGlnGlyAspLeuSerLeuGlyThrProAspGlnLysProPheGlnGly

1090 1100 1110 1120 1130 1140
CTGGAGAACCGTACCCAGCAGCCTTCGCTCACTCCACTATCCACTATTAAAGCCTTCGCC
LeuGluAsnArgThrGlnGlnProSerLeuThrProLeuSerThrIleLysAlaPheAla

1150 1160 1170 1180 1190 1200
ACTCAGTCGGGCTCCCAGGACTTAAAGGCTCTTAATACCACCTACCAATCCCAGCTCATC
ThrGlnSerGlySerGlnAspLeuLysAlaLeuAsnThrThrTyrGlnSerGlnLeuIle

1210 1220 1230 1240 1250 1260
AAACCCAGCCGCATGCGCAAGTACCCCAACCGGCCCAGCAAGACACCCCCCCATGAACGC
LysProSerArgMetArgLysTyrProAsnArgProSerLysThrProProHisGluArg

FIG. 1.3

1270 1280 1290 1300 1310 1320
CCATATGCTTGCCCTGTCGAGTCCTGCGATCGCCGCTTTTCTCGCTCGGATGAGCTTACC
ProTyrAlaCysProValGluSerCysAspArgArgPheSerArgSerAspGluLeuThr

* * FINGER 1
1330 1340 1350 1360 1370 1380
CGCCATATCCGCATCCACACAGGCCAGAAGCCCTTCCAGTGTCGAATCTGCATGCGTAAC
ArgHisIleArgIleHisThrGlyGlnLysProPheGlnCysArgIleCysMetArgAsn

* * * *
1390 1400 1410 1420 1430 1440
TTCAGTCGTAGTGACCACCTTACCACCCACATCCGCACCCACACAGGCGAGAAGCCTTTT
PheSerArgSerAspHisLeuThrThrHisIleArgThrHisThrGlyGluLysProPhe

FINGER 2 * * *
1450 1460 1470 1480 1490 1500
GCCTGTGACATTTGTGGGAGGAAGTTTGCCAGGAGTGATGAACGCAAGAGGCATACCAAA
AlaCysAspIleCysGlyArgLysPheAlaArgSerAspGluArgLysArgHisThrLys

* * FINGER 3 *
1510 1520 1530 1540 1550 1560
ATCCATTTAAGACAGAAGGACAAGAAAGCAGACAAAAGTGTGGTGGCCTCCCCGGCTGCC
IleHisLeuArgGlnLysAspLysLysAlaAspLysSerValValAlaSerProAlaAla

*
1570 1580 1590 1600 1610 1620
TCTTCACTCTCTTCTTACCCATCCCCAGTGGCTACCTCCTACCCATCCCCTGCCACCACC
SerSerLeuSerSerTyrProSerProValAlaThrSerTyrProSerProAlaThrThr

1630 1640 1650 1660 1670 1680
TCATTCCCATCCCCTGTGCCCACTTCCTACTCCTCTCCTGGCTCCTCCACCTACCCATCT
SerPheProSerProValProThrSerTyrSerSerProGlySerSerThrTyrProSer

FIG. 1.4

```
       1690      1700      1710      1720      1730      1740
CCTGCGCACAGTGGCTTCCCGTCGCCGTCAGTGGCCACCACCTTTGCCTCCGTTCCACCT
ProAlaHisSerGlyPheProSerProSerValAlaThrThrPheAlaSerValProPro

       1750      1760      1770      1780      1790      1800
GCTTTCCCCACCCAGGTCAGCAGCTTCCCGTCTGCGGGCGTCAGCAGCTCCTTCAGCACC
AlaPheProThrGlnValSerSerPheProSerAlaGlyValSerSerSerPheSerThr

       1810      1820      1830      1840      1850      1860
TCAACTGGTCTTTCAGACATGACAGCGACCTTTTCTCCCAGGACAATTGAAATTTGCTAA
SerThrGlyLeuSerAspMetThrAlaThrPheSerProArgThrIleGluIleCys

       1870      1880      1890      1900      1910      1920
AGGGAATAAAAGAAAGCAAAGGGAGAGGCAGGAAAGACATAAAAGCACAGGAGGGAAGAG

       1930      1940      1950      1960      1970      1980
ATGGCCGCAAGAGGGGCCACCTCTTAGGTCAGATGGAAGATCTCAGAGCCAAGTCCTTCT

       1990      2000      2010      2020      2030      2040
ACTCACGAGTAGAAGGACCGTTGGCCAACAGCCCTTTCACTTACCATCCCTGCCTCCCCC

       2050      2060      2070      2080      2090      2100
GTCCTGTTCCCTTTGACTTCAGCTGCCTGAAACAGCCATGTCCAAGTTCTTCACCTCTAT
```

FIG. 1.5

WO 94/23030


```
       2110      2120      2130      2140      2150      2160
CCAAAGGACTTGATTTGCATGGTATTGGATAAATCATTTCAGTATCCTCTCCATCACATG

       2170      2180      2190      2200      2210      2220
CCTGGCCCTTGCTCCCTTCAGCGCTAGACCATCAAGTTGGCATAAAGAAAAAAAAATGGG

       2230      2240      2250      2260      2270      2280
TTTGGGCCCTCAGAACCCTGCCCTGCATCTTTGTACAGCATCTGTGCCATGGATTTTGTT
       2290      2300      2310      2320      2330      2340
TTCCTTGGGGTATTCTTGATGTGAAGATAATTTGCATACTCTATTGTATTATTTGGAGTT

       2350      2360      2370      2380      2390      2400
AAATCCTCACTTTGGGGGAGGGGGGAGCAAAGCCAAGCAAACCAATGATGATCCTCTATT

       2410      2420      2430      2440      2450      2460
TTGTGATGACTCTGCTGTGACATTAGGTTTGAAGCATTTTTTTTTTCAAGCAGCAGTCCT

       2470      2480      2490      2500      2510      2520
AGGTATTAACTGGAGCATGTGTCAGAGTGTTGTTCCGTTAATTTTGTAAATACTGGCTCG
```

FIG. 1.6

SUBSTITUTE SHEET (RULE 26)

PCT/US94/02611

```
   2530      2540      2550      2560      2570      2580
ACTGTAACTCTCACATGTGACAAAGTATGGTTTGTTTGGTTGGGTTTTGTTTTTGAGAAT

   2590      2600      2610      2620      2630      2640
TTTTTTGCCCGTCCCTTTGGTTTCAAAAGTTTCACGTCTTGGTGCCTTTTGTGTGACACG

   2650      2660      2670      2680      2690      2700
CCTTCCGATGGCTTGACATGCGCAGATGTGAGGGACACGCTCACCTTAGCCTTAAGGGGG
   2710      2720      2730      2740      2750      2760
TAGGAGTGATGTGTTGGGGGAGGCTTGAGAGCAAAAACGAGGAAGAGGGCTGAGCTGAGC

   2770      2780      2790      2800      2810      2820
TTTCGGTCTCCAGAATGTAAGAAGAAAAAATTTAAACAAAAATCTGAACTCTCAAAAGTC
```

FIG. 1.7


```
   2830      2840      2850      2860      2870      2880
TATTTTTCTAAACTGAAAATGTAAATTTATACATCTATTCAGGAGTTGGAGTGTTGTGGT

   2890      2900      2910      2920      2930      2940
TACCTACTGAGTAGGCTGCAGTTTTTGTATGTTATGAACATGAAGTTCATTATTTTGTGG

   2950      2960      2970      2980      2990      3000
TTTTATTTTACTTTGTACTTGTGTTTGCTTAAACAAAGTAACCTGTTTGGCTTATAAACA

   3010      3020      3030      3040      3050      3060
CATTGAATGCGCTCTATTGCCCATGGGATATGTGGTGTGTATCCTTCAGAAAAATTAAAA

   3070      3080
GGAAAAATAAAAAAAAAAAAAAAAAA
```

FIG. 1.8

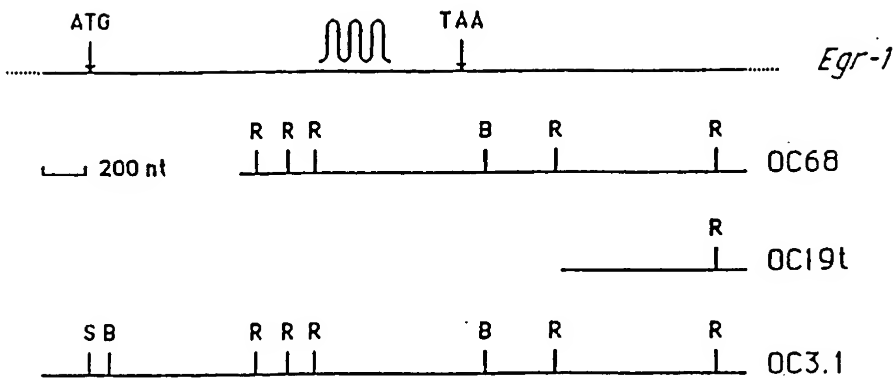


FIG. 2

"Zinc finger" consensus sequence	T G E R/K P F/Y X C X X X X C X X X F X X X X X L X X H X X X H
Murine Egr-1	P H E R P Y A C P V E S C D R R F S R S D E L T R H I R I H
	T G Q K P F Q C - - R I C M R N F S R S D H L T T H I R T H
	T G E K P F A C - - D I C G R K F A R S D E R K R H T K I H
Drosophila Kruppel	S R D K S F T C K I - - C S R S F G Y K H V L Q N H E R T H
	T G E K P F E C P E - - C D K R F T R D H H L K T H M R L H
	T G E K P Y H C S H - - C D R Q F V Q V A N L R R H L R V H
	T G E R P Y T C E I - - C D G K F S D S N Q L K S H M L V H
Xenopus TFIIIA (finger 2)	T G E K P F P C K E E G C E K G F T S L H H L T R H S L T H

FIG. 3

```
         10        20        30        40        50        60
TTTTTTTTTTTGGTGTGTGTGGTGGTTGTTTTTAAGTGTGGAGGGCAAAAGGAGATACCA

         70        80        90       100       110       120
TCCCAGGCTCAGTCCAACCCCTCTCCAAAACNGTGTCTTTTCTGACACTCCAGGTAGCGA

        130       140       150       160       170       180
GGGAGTTGGGTCTCCAGGTTGTGCGAGGAGCAAATGATGACCGCCAAGGCCGTAGACAAA
                                   MetMetThrAlaLysAlaValAspLys

        190       200       210       220       230       240
ATCCCAGTAACTCTCAGTGGTTTTGTGCACCAGCTGTCTGACAACATCTACCCGGTGGAG
IleProValThrLeuSerGlyPheValHisGlnLeuSerAspAsnIleTyrProValGlu

        250       260       270       280       290       300
GACCTCGCCGCCACGTCGGTGACCATCTTTCCCAATGCCGAACTGGGAGGCCCCTTTGAC
AspLeuAlaAlaThrSerValThrIlePheProAsnAlaGluLeuGlyGlyProPheAsp

        310       320       330       340       350       360
CAGATGAACGGAGTGGCCGGAGATGGCATGATCAACATTGACATGACTGGAGAGAAGAGG
GlnMetAsnGlyValAlaGlyAspGlyMetIleAsnIleAspMetThrGlyGluLysArg
```

FIG. 4.1


```
        370       380       390       400       410       420
TCGTTGGATCTCCCATATCCCAGCAGCTTTGCTCCCGTCTCTGCACCTAGAAACCAGACC
SerLeuAspLeuProTyrProSerSerPheAlaProValSerAlaProArgAsnGlnThr

        430       440       450       460       470       480
TTCACTTACATGGGCAAGTTCTCCATTGACCCACAGTACCCTGGTGCCAGCTGCTACCCA
PheThrTyrMetGlyLysPheSerIleAspProGlnTyrProGlyAlaSerCysTyrPro

        490       500       510       520       530       540
GAAGGCATAATCAATATTGTGAGTGCAGGCATCTTGCAAGGGGTCACTTCCCCAGCTTCA
GluGlyIleIleAsnIleValSerAlaGlyIleLeuGlnGlyValThrSerProAlaSer

        550       560       570       580       590       600
ACCACAGCCTCATCCAGCGTCACCTCTGCCTCCCCCAACCCACTGGCCACAGGACCCCTG
ThrThrAlaSerSerSerValThrSerAlaSerProAsnProLeuAlaThrGlyProLeu

        610       620       630       640       650       660
GGTGTGTGCACCATGTCCCAGACCCAGCCTGACCTGGACCACCTGTACTCTCCGCCACCG
GlyValCysThrMetSerGlnThrGlnProAspLeuAspHisLeuTyrSerProProPro

        670       680       690       700       710       720
CCTCCTCCTCCTTATTCTGGCTGTGCAGGAGACCTCTACCAGGACCCTTCTGCGTTCCTG
ProProProProTyrSerGlyCysAlaGlyAspLeuTyrGlnAspProSerAlaPheLeu
```

FIG. 4.2

```
     730       740       750       760       770       780
TCAGCAGCCACCACCTCCACCTCTTCCTCTCTGGCCTACCCACCACCTCCTTCCTATCCA
SerAlaAlaThrThrSerThrSerSerSerLeuAlaTyrProProProProSerTyrPro

     790       800       810       820       830       840
TCCCCCAAGCCAGCCACGGACCCAGGTCTCTTCCCAATGATCCCAGACTATCCTGGATTC
SerProLysProAlaThrAspProGlyLeuPheProMetIleProAspTyrProGlyPhe

     850       860       870       880       890       900
TTTCCATCTCAGTGCCAGAGAGACCTACATGGTACAGCTGGCCCAGACCGTAAGCCCTTT
PheProSerGlnCysGlnArgAspLeuHisGlyThrAlaGlyProAspArgLysProPhe

     910       920       930       940       950       960
CCCTGCCCACTGGACACCCTGCGGGTGCCCCCTCCACTCACTCCACTCTCTACAATCCGT
ProCysProLeuAspThrLeuArgValProProProLeuThrProLeuSerThrIleArg

     970       980       990      1000      1010      1020
AACTTTACCCTGGGGGGCCCCAGTGCTGGGATGACCGGACCAGGGGCCAGTGGAGGCAGC
AsnPheThrLeuGlyGlyProSerAlaGlyMetThrGlyProGlyAlaSerGlyGlySer

    1030      1040      1050      1060      1070      1080
GAGGGACCCCGGCTGCCTGGTAGCAGCTCAGCAGCAGCAGCAGCCGCCGCCGCCGCCGCC
GluGlyProArgLeuProGlySerSerSerAlaAlaAlaAlaAlaAlaAlaAlaAlaAla
```

FIG. 4.3


```
    1090      1100      1110      1120      1130      1140
TATAACCCACACCACCTGCCACTGCGGCCCATTCTGAGGCCTCGCAAGTACCCCAACAGA
TyrAsnProHisHisLeuProLeuArgProIleLeuArgProArgLysTyrProAsnArg

    1150      1160      1170      1180      1190      1200
CCCAGCAAGACGCCGGTGCACGAGAGGCCCTACCCGTGCCCAGCAGAAGGCTGCGACCGG
ProSerLysThrProValHisGluArgProTyrProCysProAlaGluGlyCysAspArg

    1210      1220      1230      1240      1250      1260
CGGTTCTCCCGCTCTGACGAGCTGACACGGCACATCCGAATCCACACTGGGCATAAGCCC
ArgPheSerArgSerAspGluLeuThrArgHisIleArgIleHisThrGlyHisLysPro

    1270      1280      1290      1300      1310      1320
TTCCAGTGTCGGATCTGCATGCGCAACTTCAGCCGCAGTGACCACCTCACCACCCATATC
PheGlnCysArgIleCysMetArgAsnPheSerArgSerAspHisLeuThrThrHisIle

    1330      1340      1350      1360      1370      1380
CGCACCCACACCGGTGAGAAGCCCTTCGCCTGTGACTACTGTGGCCGAAAGTTTGCCCGG
ArgThrHisThrGlyGluLysProPheAlaCysAspTyrCysGlyArgLysPheAlaArg

    1390      1400      1410      1420      1430      1440
AGTGATGAGAGGAAGCGCCACACCAAGATCCACCTGAGACAGAAAGAGCGGAAAAGCAGT
SerAspGluArgLysArgHisThrLysIleHisLeuArgGlnLysGluArgLysSerSer
```

FIG. 4.4

```
      1450      1460      1470      1480      1490      1500
GCCCCCTCTGCATCGGTGCCAGCCCCCTCTACAGCCTCCTGCTCTGGGGGCGTGCAGGCC
AlaProSerAlaSerValProAlaProSerThrAlaSerCysSerGlyGlyValGlnAla

      1510      1520      1530      1540      1550      1560
TGGGGGTACCCTGTGCAGCAGTAACAGCAGCAGTCTTGGCGGAGGGCCGCTCGCCCCTTG
TrpGlyTyrProValGlnGlnEnd

      1570      1580      1590      1600      1610      1620
CTCCTCTCGGACCCGGACACCTTGAGATGAGACTCAGGCTGATACACCAGCTCCCAAAGG

      1630      1640      1650      1660      1670      1680
TCCCGGAGGCCCTTTGTCCACTGGAGCTGCACAACAAACACTACCACCCTTTCCTGTCCC

      1690      1700      1710      1720      1730      1740
TCTCTCCCTTTGTTGGGCAAAGGGCTTTGGTGGAGCTAGCACTGCCCCCTTTCCACCTAG

      1750      1760      1770      1780      1790      1800
AAGCAGGTTCTTCCTAAAACTTAGCCCATTCTAGTCTCTCTTAGGTGAGTTGACTATCAA
```

FIG. 4.5


```
      1810      1820      1830      1840      1850      1860
CCCAAGGCAAAGGGGAGGCTCAGAAGGAGGTGGTGTGGGGATCCCCTGGCCAAGAGGGCT

      1870      1880      1890      1900      1910      1920
GAGGTCTGACCCTGCTTTAAAGGGTTGTTTGACTAGGTTTTGCTACCCCACTTCCCCTTA

      1930      1940      1950      1960      1970      1980
TTTTGACCCATCACAGGTTTTTGACCCTGGATGTCAGAGTTGATCTAAGACGTTTTCTAC

      1990      2000      2010      2020      2030      2040
AATAGGTTGGGAGATGCTGATCCCTTCAAGTGGGGACAGCAAAAAGACAAGCAAAACTGA

      2050      2060      2070      2080      2090      2100
TGTGCACTTTATGGCTTGGGACTGATTTGGGGGACATTGTACAGTGAGTGAAGTATAGCC

      2110      2120      2130      2140      2150      2160
TTTATGCCACACTCTGTGGCCCTAAAATGGTGAATCAGAGCATATCTAGTTGTCTCAACC
```

FIG. 4.6

```
     2170      2180      2190      2200      2210      2220
CTTGAAGCAATATGTATTATATACTCAGAGAACAGAAGTGCAATGTGATGGGAGGAACGT

     2230      2240      2250      2260      2270      2280
AGCAATATCTGCTCCTTTTCGAGTTGTTTGAGAAATGTAGGCTATTTTTTCAGTGTATAT
     2290      2300      2310      2320      2330      2340
CCACTCAGATTTTGTGTATTTTTGATGTACCCACACTGTTCTCTAAATTCTGAATCTTTG

     2350      2360      2370      2380      2390      2400
GGAAAAAATGTAAAGCATTTATGATCTCAGAGGTTAACTTATTTAAGGGGGATGTACATA

     2410      2420      2430      2440      2450      2460
TTCTCTGAAACTAGGATGCATGCAATTGTGTTGGAAGTGTCCTTGGTCGCCTTGTGTGAT

     2470      2480      2490      2500      2510      2520
GTAGACAAATGTTACAAGGCTGCATGTAAATGGGTTGCCTTATTATGGAGAAAAAAATCA
```

FIG. 4.7


```
     2530      2540      2550      2560      2570      2580
CTCCCTGAGTTTAGTATGGCTGTATATTTATGCCTATTAATATTTCAAATTTTTTTTTAG

     2590      2600      2610      2620      2630      2640
AGTATATTTTTGTATGCTTTGTTTTGTGACTTAAAAGTGTTACCTTTGTAGTCAAATTTC

     2650      2660      2670      2680      2690      2700
AGATAAGAATGTACATAATGTTACCGGAGCTGANNNTTGTTTGGTCATTAGCTCTTAATA
     2710      2720      2730      2740      2750      2760
GTTGTGAAAAAATAAATCTATTCTAACGCAAAACCACTAACTGAAGTTCAGATATAATGG

     2770      2780      2790      2800      2810
ATGGTTTGTGACTATAGTGTAAATAAATACTTTTCAACAAAAAAAAAAAAAAAAAAA
```

FIG. 4.8

-935 -876
ACGGAGGGAA TAGCCTTTCG ATTCTGGGTG GTGCATTGGA AGCCCCAGGC TCTAAAACCC
-875 -816
CCAACCTACT GACTGGTGGC CGAGTATGCA CCCGACTGCT AGCTAGGCAG TGTCCCAAGA
-815 -756
ACCAGTAGCC AAATGTCTTG GCCTCAGTTT TCCCGGTGAC ACCTGGAAAG TGACCCTGCC
-755 -696
ATTAGTAGAG GCTCAGGTCA GGGCCCCGCC TCTCCTGGGC GGCCTCTGCC CTAGCCCGCC
-695 -636
CTGCCGCTCC TCCTCTCCGC AGGCTCGCTC CCACGGTCCC CGAGGTGGGC GGGTGAGCCC
-635 -576
AGGATGACGG CTGTAGAACC CCGGCCTGAC TCGCCCTCGC CCCCGCGCCG GGCCTGGGCT
-575 -516
TCCCTAGCCC AGCTCGCACC CGGGGGCCGT CGGAGCCGCC GCGCGCCCAG CTCTACGCGC
-515 -456
CTGGCCCTCC CCACGCGGGC GTCCCCGACT CCCGCGCGCG CTCAGGCTCC CAGTTGGGAA
-455 -396
CCAAGGAGGG GGAGGATGGG GGGGGGGGTG TGCGCCGACC CGGAAACGCC ATATAAGGAG
-395 -336
CAGGAAGGAT CCCCCGCCGG AACAGACCTT ATTTGGGCAG CGCCTTATAT GGAGTGGCCC

FIG. 5.1

-335 -276
AATATGGCCC TGCCGCTTCC GGCTCTGGGA GGAGGGGCGA GCGGGGGTTG GGGCGGGGGC
-275 -216
AAGCTGGGAA CTCCAGGCGC CTGGCCCGGG AGGCCACTGC TGCTGTTCCA ATACTAGGCT
SmaI
-215 -156
TTCCAGGAGC CTGAGCGCTC GCGATGCCGG AGCGGGTCGC AGGGTGGAGG TGCCCACCAC
-155 -96
TCTTGGATGG GAGGGCTTCA CGTCACTCCG GGTCCTCCCG GCCGGTCCTT CCATATTAGG
-95 -36
GCTTCCTGCT TCCCATATAT GGCCATGTAC GTCACGGCGG AGGCGGGCCC GTGCTGTTCC
-35 +1 +25
AGACCCTTGA AATAGAGGCC GATTCGGGGA GTCGCGAGAG ATCCCAGCGC GCAGAACTTG
+26 +85
GGGAGCCGCC GCCGCGATTC GCCGCCGCCG CCAGCTTCCG CCGCCGCAAG ATCGGCCCCT
29-mer
+86 +145
GCCCCAGCCT CCGCGGCAGC CCTGCGTCCA CCACGGGCCG CGGCTACCGC CAGCCTGGGG
+146 +205
GCCCACCTAC ACTCCCCGCA GTGTGCCCCT GCACCCCGCA TGTAACCCGG CCAACCCCCG
+206 +265
GCGAGTGTGC CCTCAGTAGC TTCGGCCCCG GGCTGCGCCC ACCACCCAAC ATCAGTTCTC
SmaI

FIG. 5.2

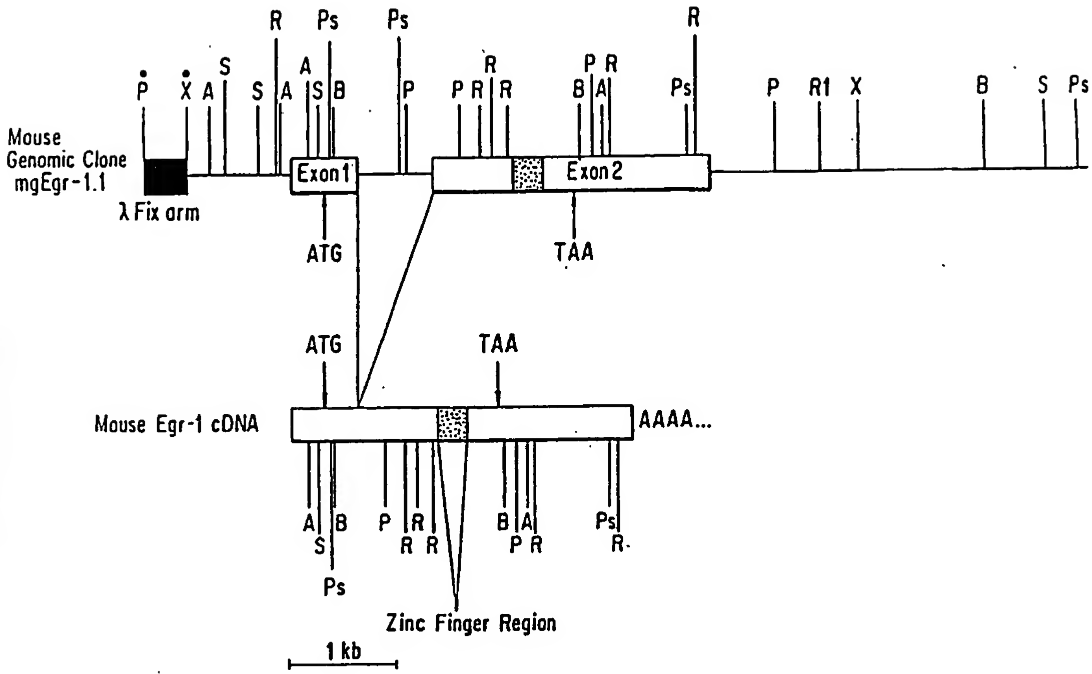


FIG. 6

Q P S L T P L S T I K A F A T Q S G S Q D L

303 K A L N T T Y Q S Q L I 315 K P S R M R K Y P N R P S K T P 330

331 P H E R P Y A C P V E S C D R R F S R S D E L T R H I R I H

361 T G Q K P F Q C - - R I C M R N F S R S D H L T T H I R T H

389 T G E K P F A C - - D I C G R X F A R S D E R K R H T K I H

417 L R Q K D K K A D K S V V A S 431

FIG. 7A

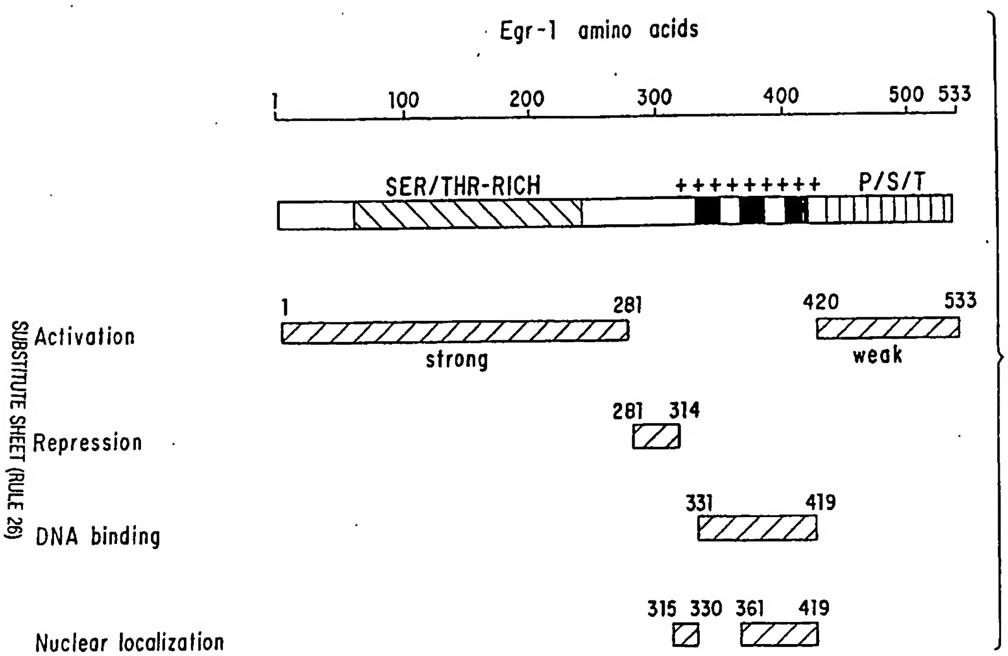


FIG. 7B

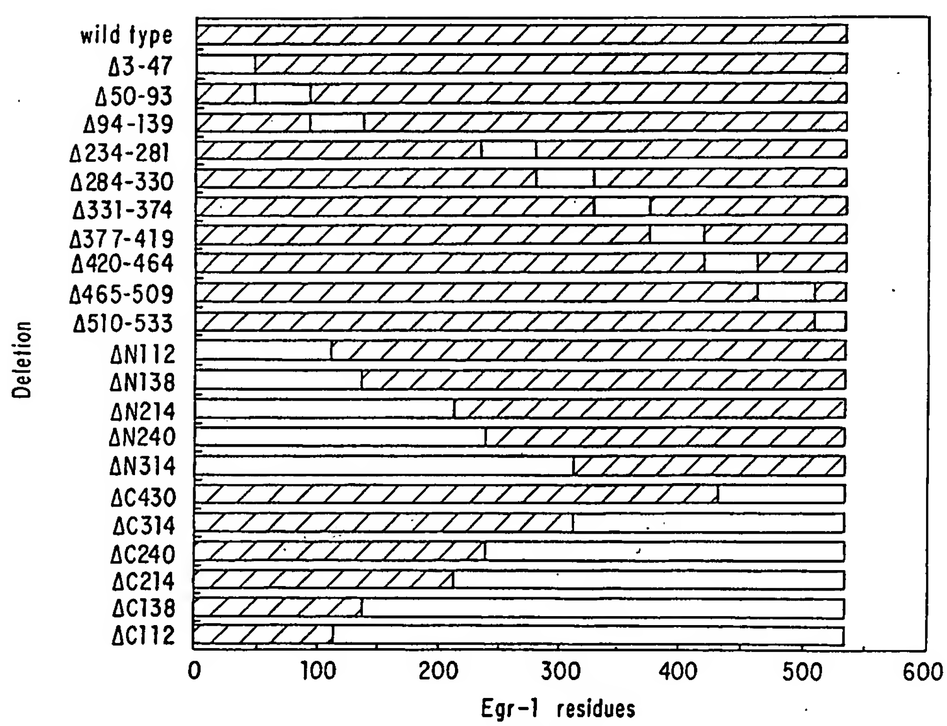
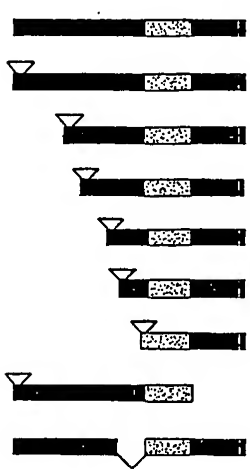


FIG. 8

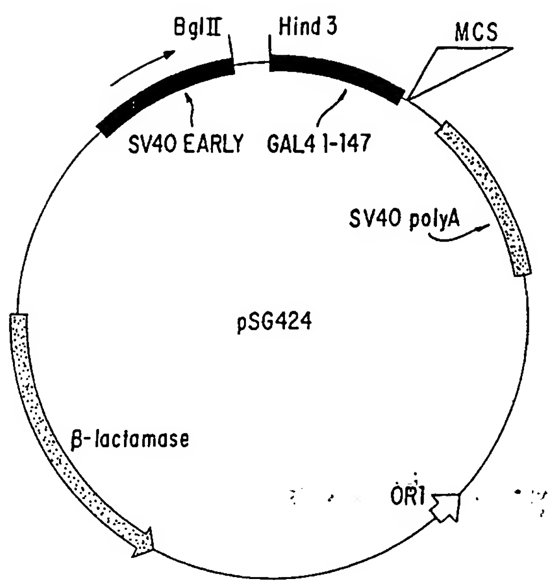
WO 94/23030 PCT/US94/02611

SUBSTITUTE SHEET (RULE 26)



	% OF WILD TYPE ACTIVATION		PROTEIN LEVELS
	HeLa	3T3	3T3
pCB6+ vector	1.62	5.03	—
pCB.Egr-1	127	89.6	+++
pCB.Egr-1.tag	100	100	+++
ΔN112	35.2	51.5	+++
ΔN138	26.4	38.1	++
ΔN214	5.59	14.7	++
ΔN240	1.62	6.31	+
ΔN314	7.82	17.2	+++++
ΔC430	19.6	97.0	+++
Δ284-330	521	39.1	ND

FIG. 9



polylinker sequence

```
Q   R   Q   L   T   V   S   P
CAA AGA CAG TTG ACT GTA TCG CCG
        143 144 145 146 147

E   L   P   G   I   R   R   R
GAA TTC CCG GGG ATC CGT CGA CGG
EcoRI   SmaI  BamHI   SalI

Y   P   G   E   F   E   L
TAC CCC GGG GAA TTC GAG CTC TAG
KpnI  SmaI  EcoRI   SacI   XbaI

ATA AGT AAT GAT TCA
            BclI
```

FIG. 10

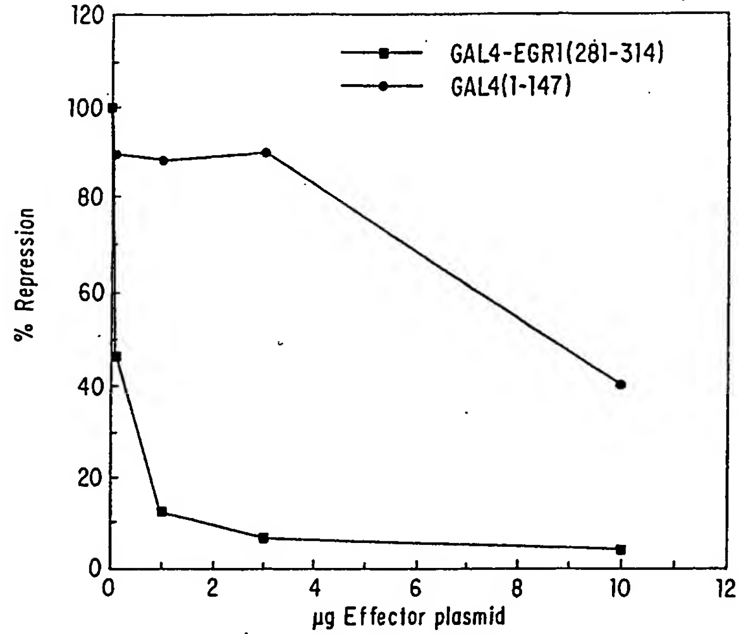


FIG. 11

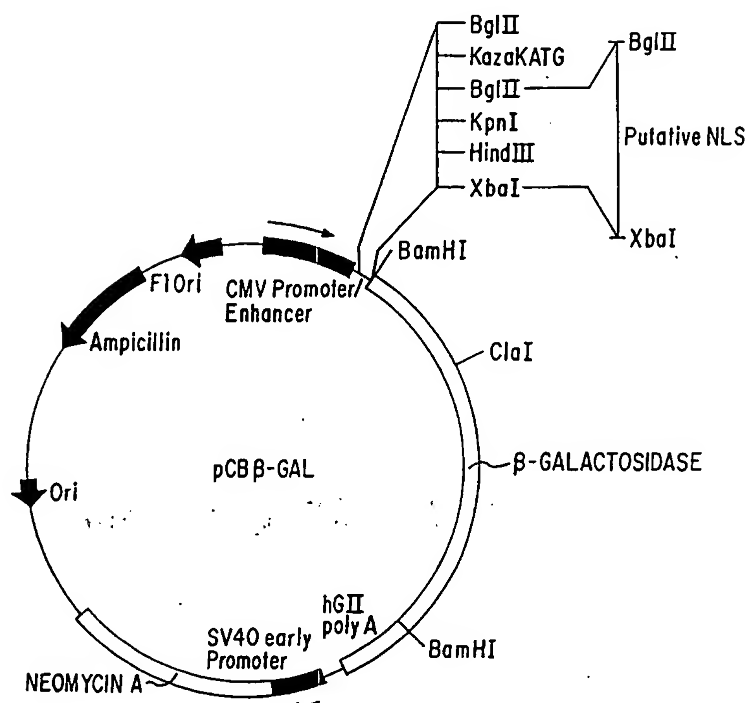


FIG. 12

SUBSTITUTE SHEET (RULE 26)

NAME	REGION OF Egr-1
pCB.β-GAL	none
pCB.315-429.β-GAL	5'basic/finger domain/3'basic
pCB.420-533.β-GAL	C-terminus
pCB.331-419.β-GAL	finger domain
pCB.315-419.β-GAL	5'basic/finger domain
pCB.331-429.β-GAL	finger domain/3'basic
pCB.315-330.β-GAL	5'basic
pCB.315-367.β-GAL	5'basic/finger 1
pCB.315-330/361-419.β-GAL	5'basic/fingers 2 and 3
pCB.315-330/361-392.β-GAL	5'basic/finger 2
pCB.315-330/389-419.β-GAL	5'basic/finger 3
pCB.315-330/389-395.β-GAL	5'basic/H-C link 3
pCB.315-330/389-395/338-363.β-GAL	5'basic/H-C link 3/finger 1
pCB.315-337/396-419.β-GAL	5'basic/H-C link 1/finger 3

CONSTRUCT

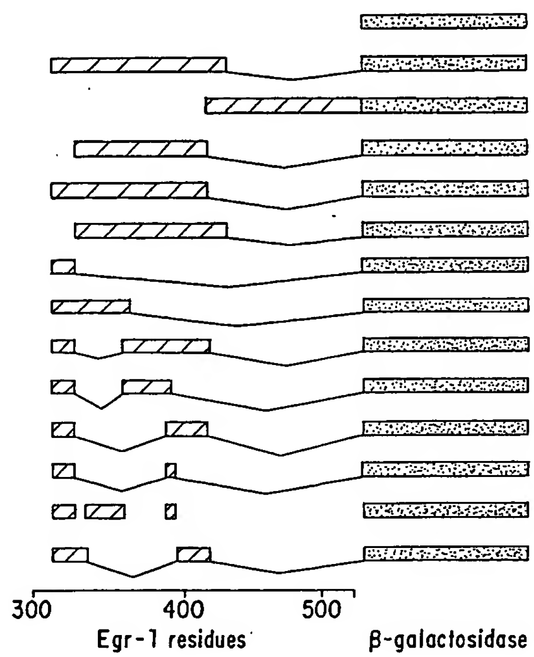


FIG. 13